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(56) Documents Cited:
CN 001418637 A
CN 001183288 A
JP 2001039868 A
US 6043276 A

(58) Field of Search:
UK CL (Edition W) A5B
INT CL[7] A61K, A61P
Other:

(54) Abstract Title: **Botanical drug or dietary supplement for use in the treatment of Hepatitis C**

(57) The present invention relates to a botanical drug or dietary supplement for use in the treatment of patients suffering from Hepatitis C virus infection. More particularly, it relates to a botanical drug consisting essentially of four botanical drug substances, optionally formulated with excipients, for use either in alleviating the symptoms of Hepatitis, particularly chronic Hepatitis C, and / or inhibiting the activity of the causative Hepatitis C virus. The botanical raw materials, botanical drug substances or botanical ingredients used are from a species of each of the genera:

(a) Silybum;
(b) Astragalus or Hedysarum;
(c) Salvia; and
(d) Schisandra.

GB 2411114 A

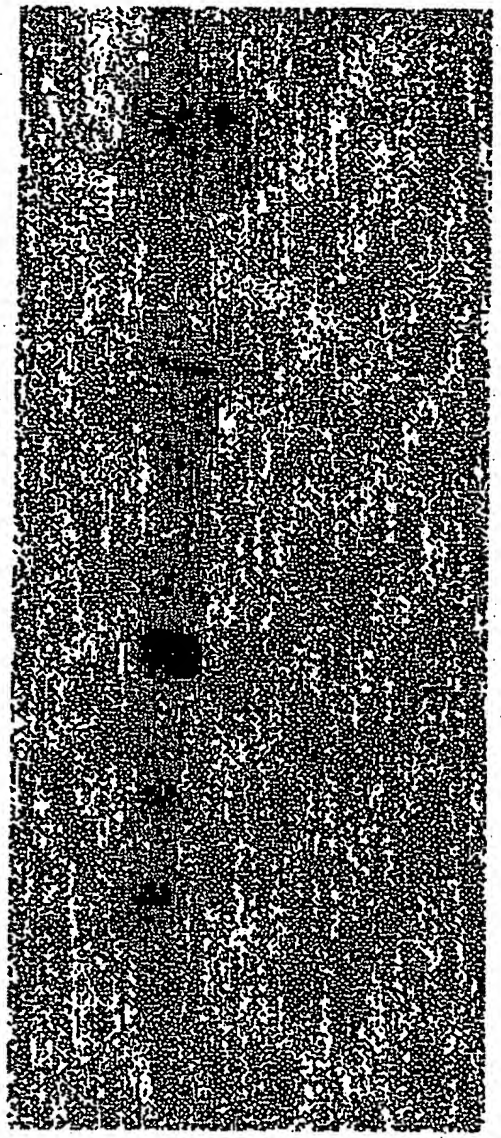


Fig. 1

Fig. 2

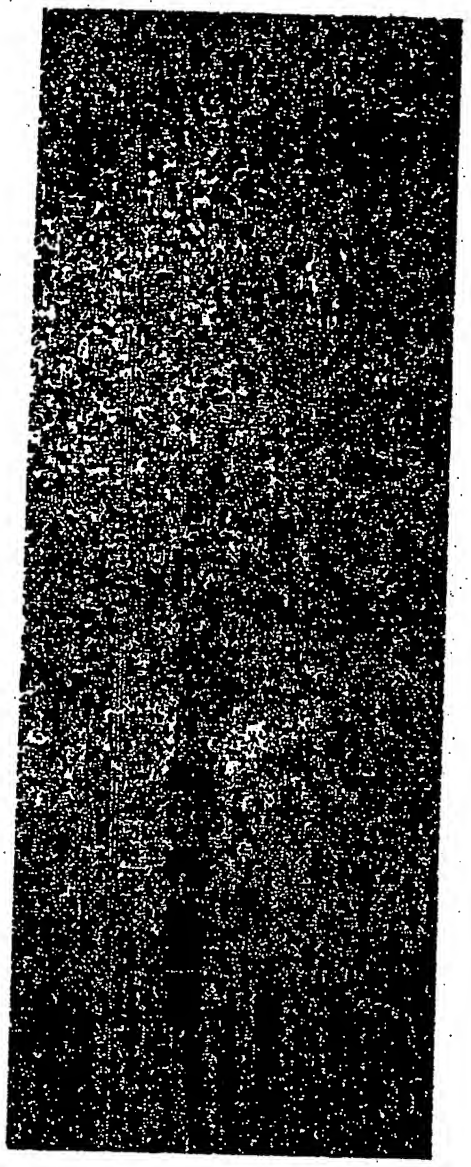
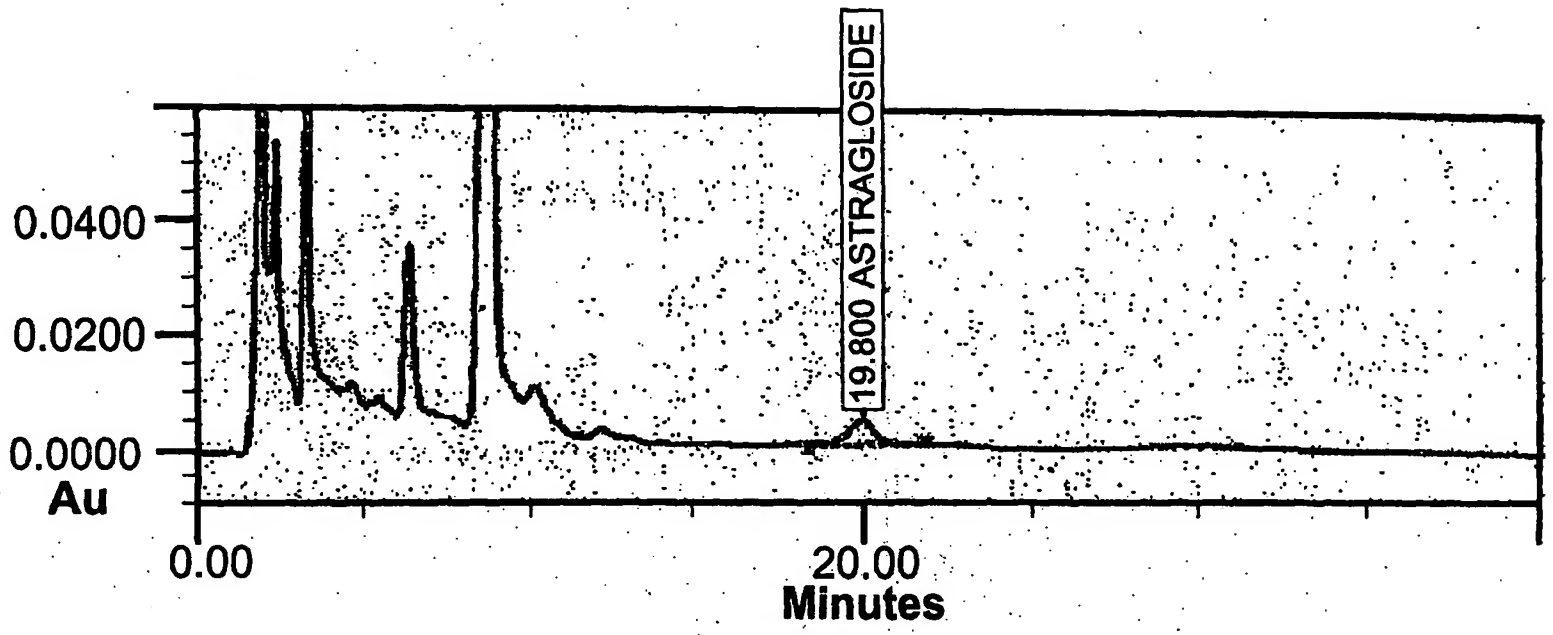


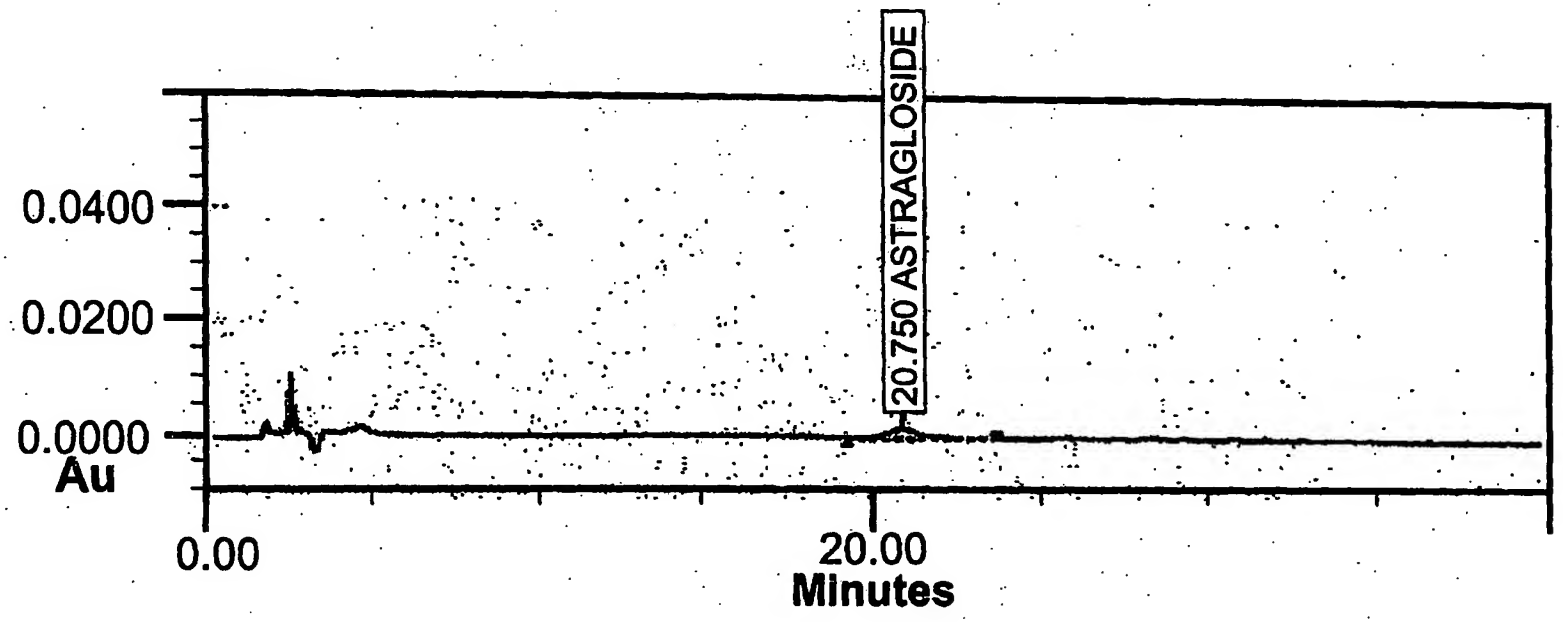
Fig. 3



Peak Results (Volume 20.00, Run Time 40.0mins)

Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Units
Astragloside	19.800	255387	4303	12.414	ug

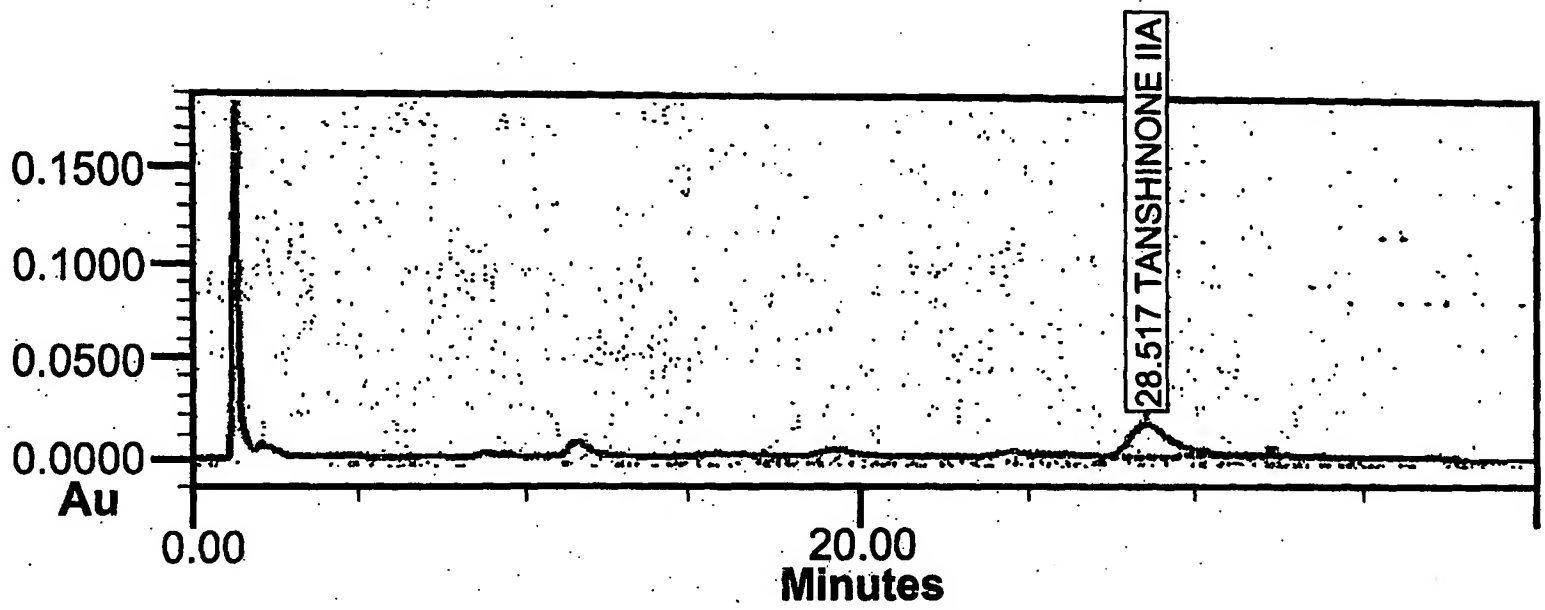
Fig. 4



Peak Results (Volume 20.00, Run Time 40.0mins)

Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Units
Astragloside	20.750	94632	1533	4.600	ug

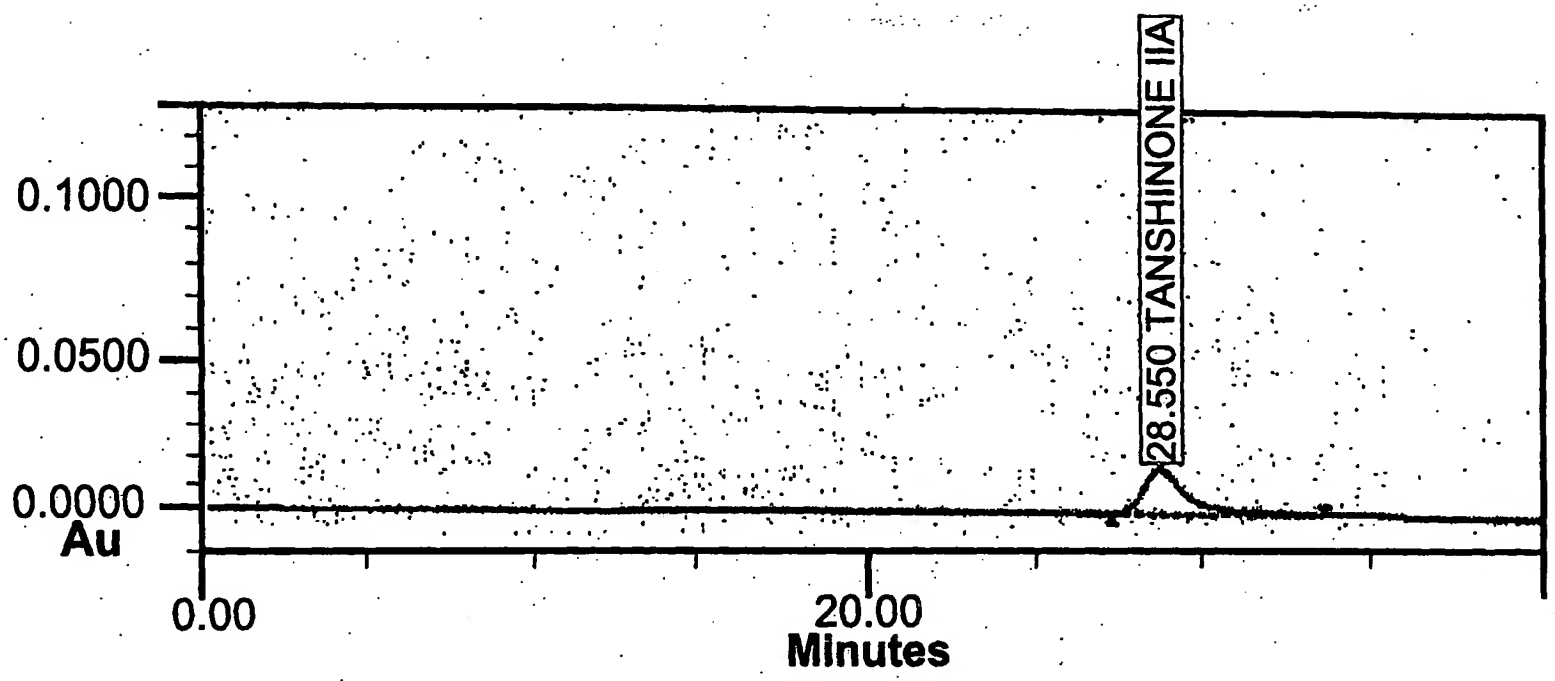
Fig. 5



Peak Results (Volume 10.00, Run Time 60.0mins)

Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Units
Tanshinone-IIA	28.517	1462676	17739	0.334	ug

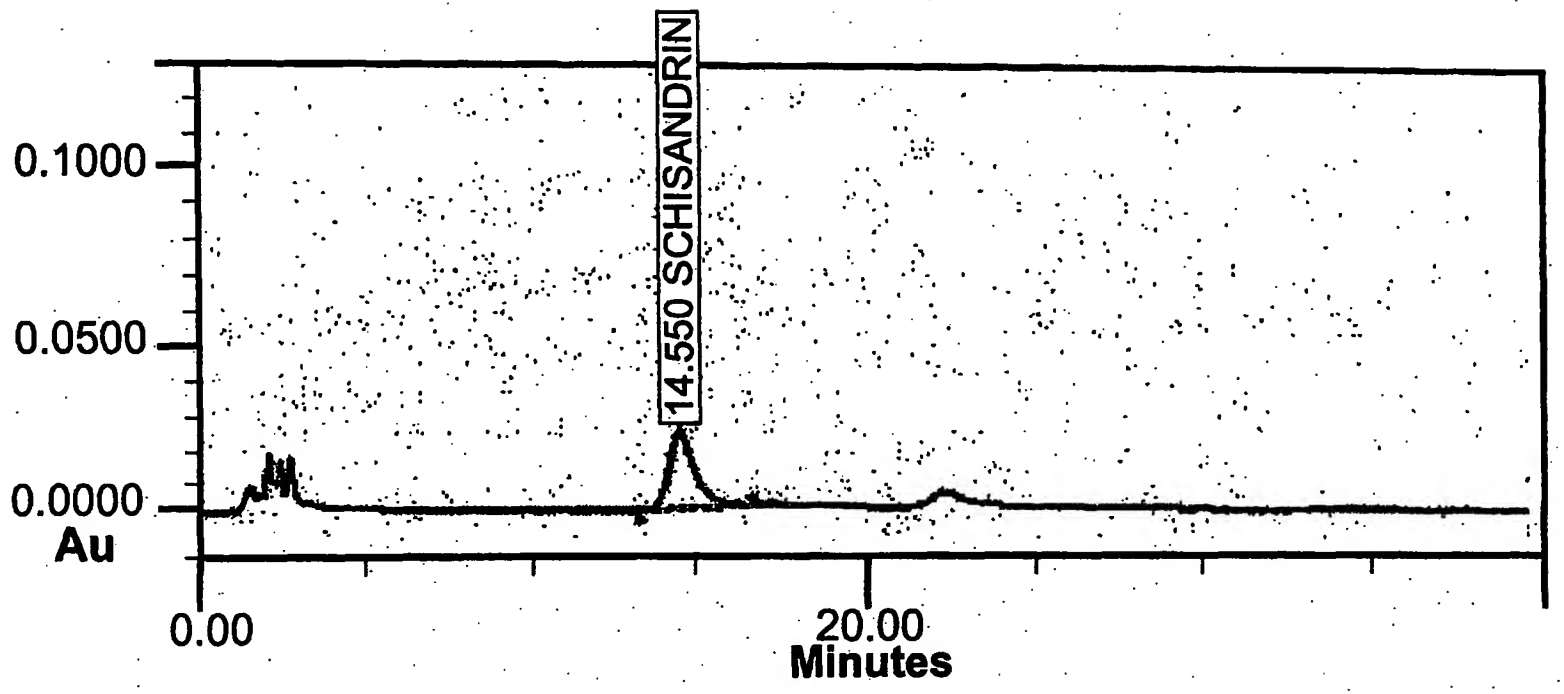
Fig. 6



Peak Results (Volume 10.00, Run Time 60.0mins)

Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Units
Tanshinone-IIA	28.550	1146871	12896	0.262	ug

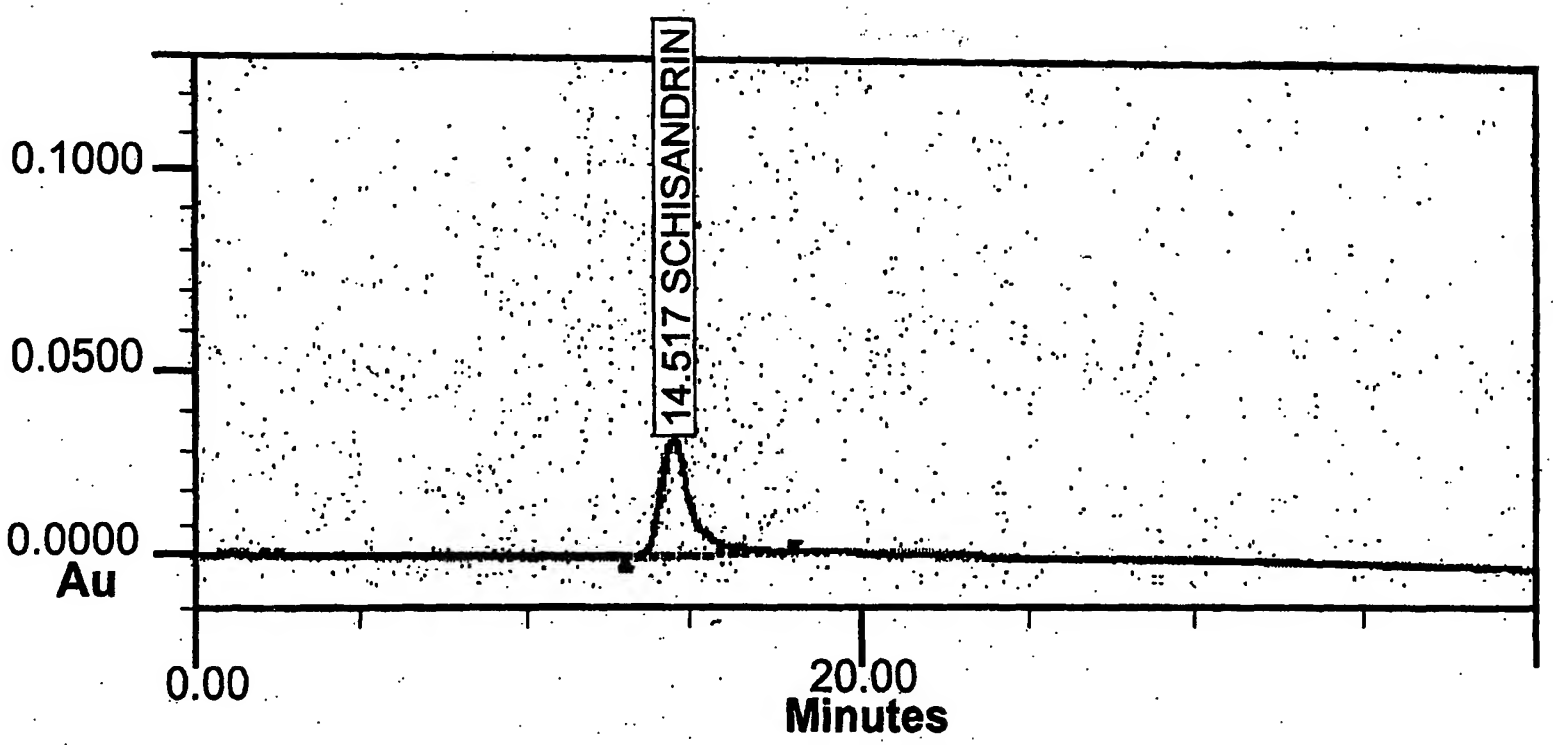
Fig. 7



Peak Results (Volume 20.00, Run Time 40.0mins)

Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Units
Schisandrin	14.550	1110494	20832	2.329	ug

Fig. 8



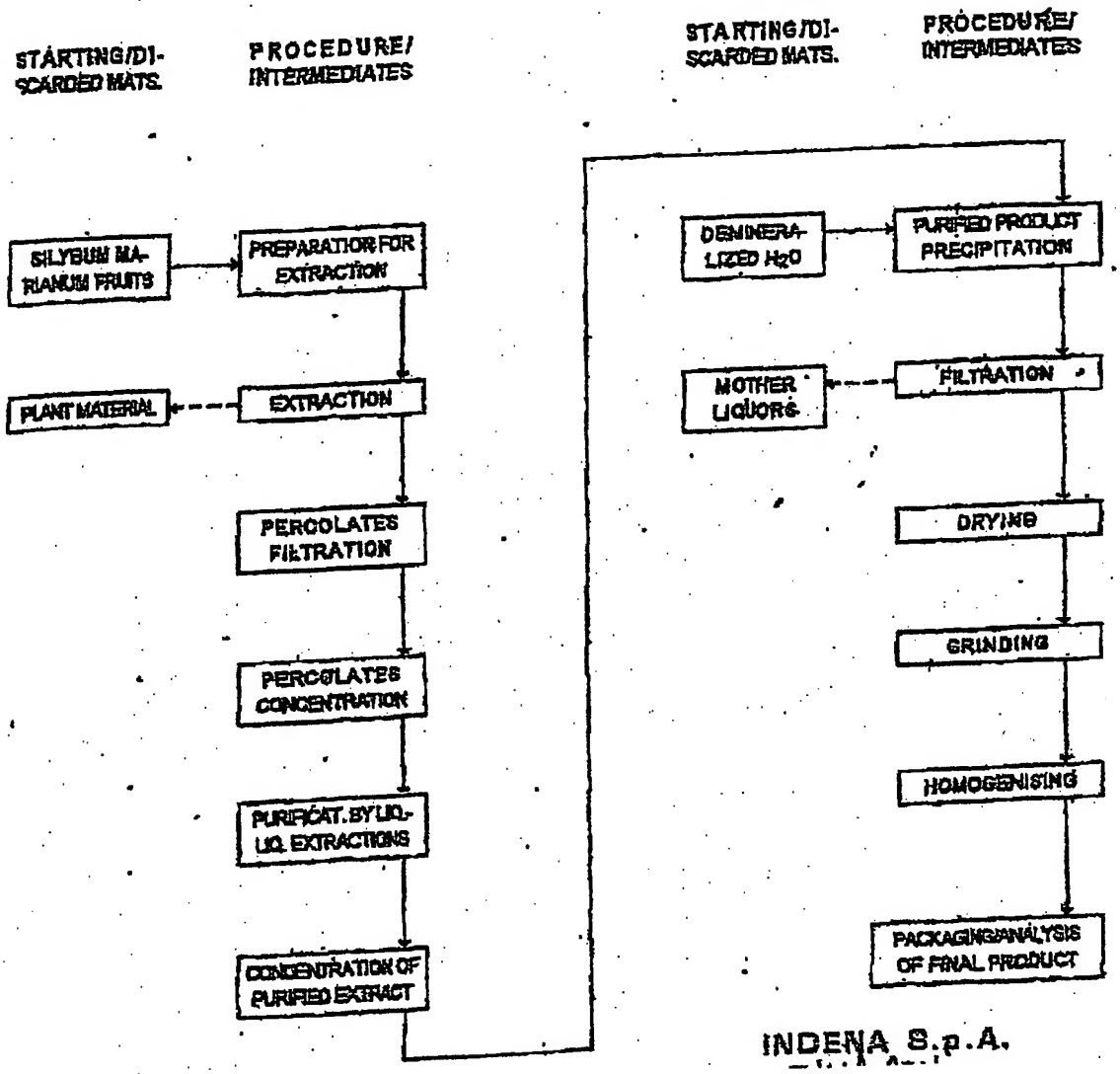
Peak Results (Volume 10.00, Run Time 40.0mins)

Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Units
Schisandrin	14.517	1487863	26508	3.120	ug

Fig. 9

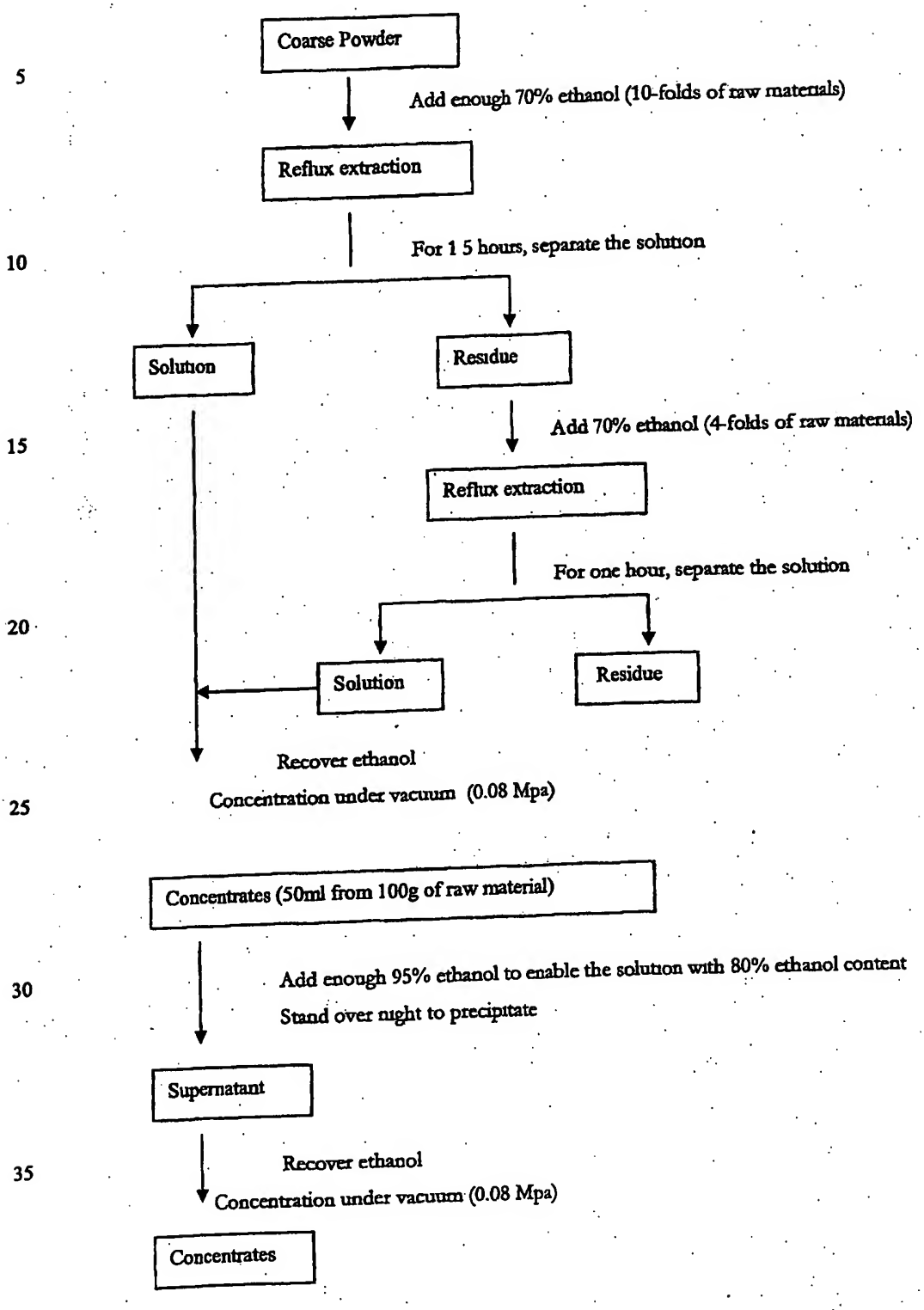
Fig 10

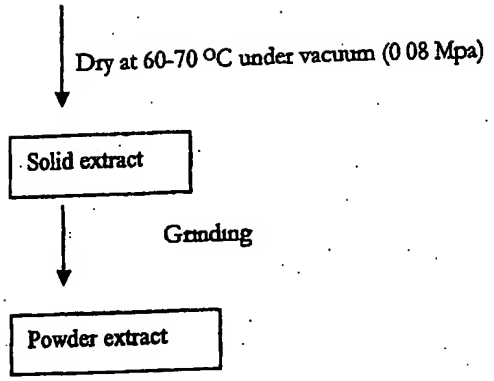
MANUFACTURING: FLOW-CHART



INDENA S.p.A.

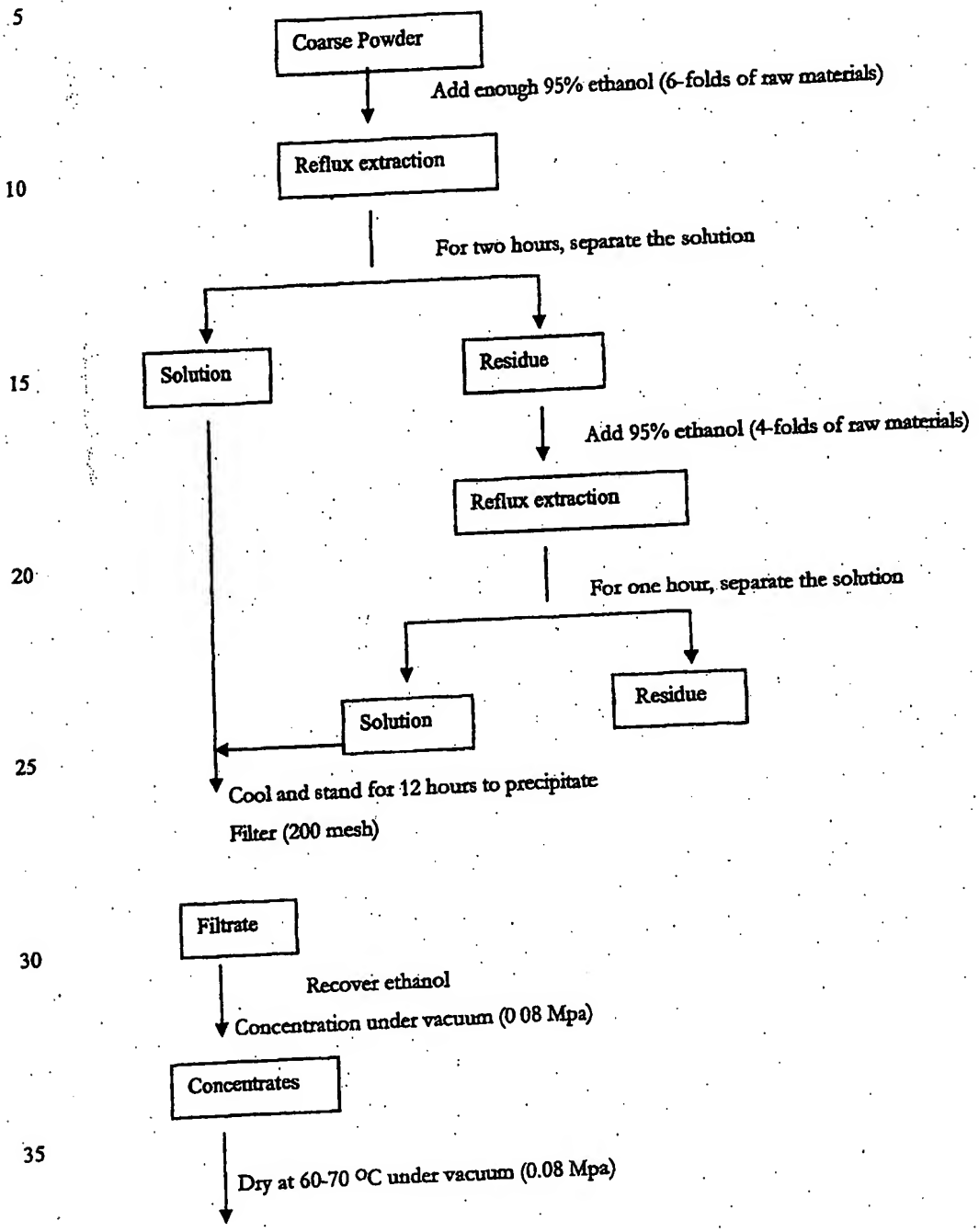
Fig 11.

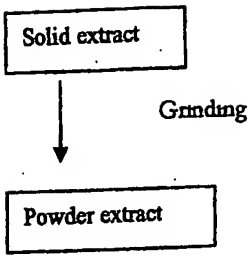

Coarse Powder
Add enough 70% ethanol (10-folds of raw materials)
Reflux extraction
For 1 5 hours, separate the solution
Solution
Residue
Add 70% ethanol (4-folds of raw materials)
Reflux extraction
For one hour, separate the solution
Solution
Residue
Recover ethanol
Concentration under vacuum (0.08 Mpa)
Concentrates (50ml from 100g of raw material)
Add enough 95% ethanol to enable the solution with 80% ethanol content
Stand over night to precipitate
Supernatant
Recover ethanol
Concentration under vacuum (0.08 Mpa)
Concentrates



(Solid yield at 11.7-13% with the content of Astragaloside IV, a chemical marker/one of the active chemicals >0.4 %)

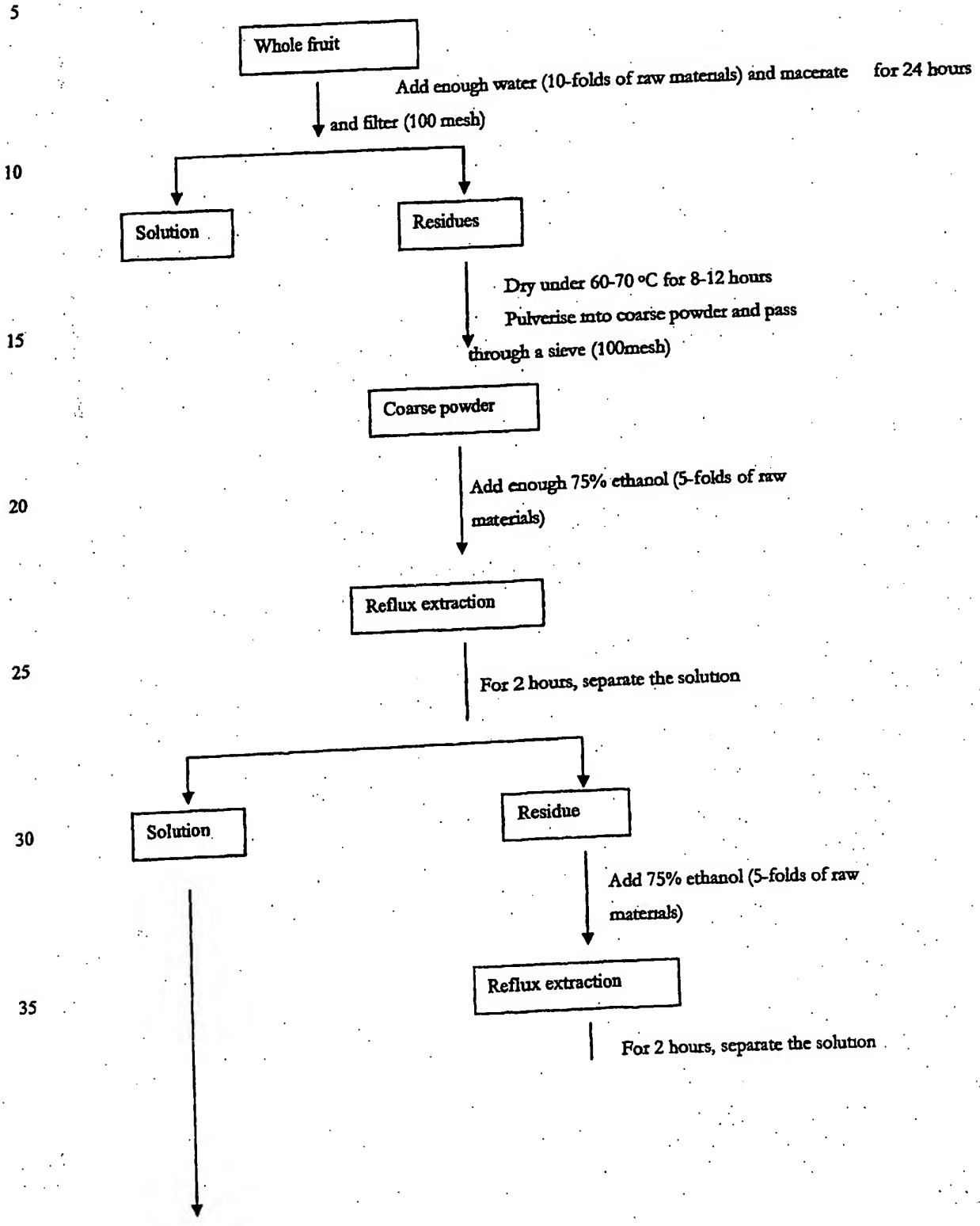
Fig 12.

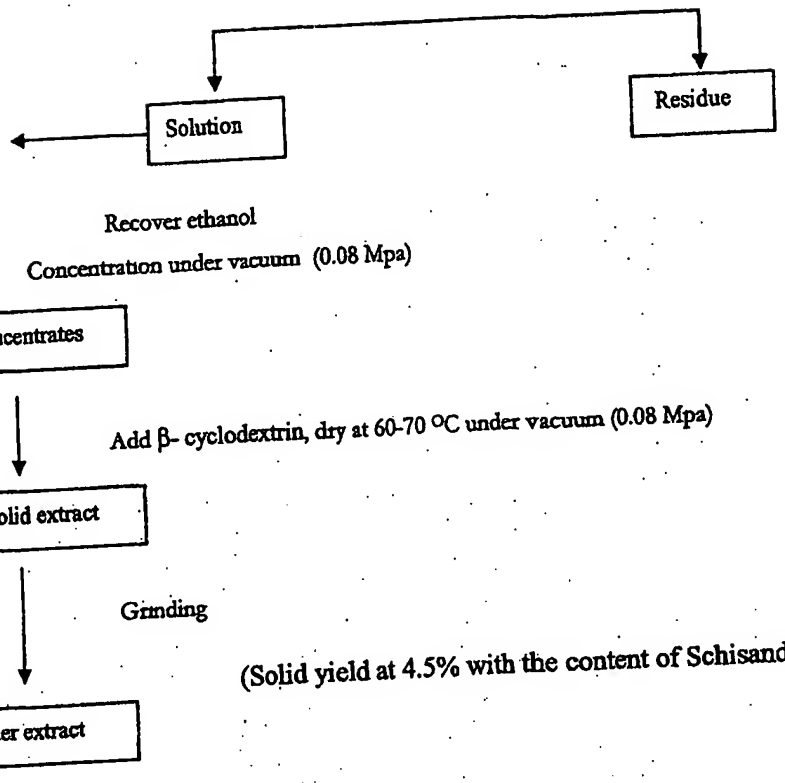

Coarse Powder
Add enough 95% ethanol (6-folds of raw materials)
Reflux extraction
For two hours, separate the solution
Solution
Residue
Add 95% ethanol (4-folds of raw materials)
Reflux extraction
For one hour, separate the solution
Solution
Residue
Cool and stand for 12 hours to precipitate
Filter (200 mesh)
Filtrate
Recover ethanol
Concentration under vacuum (0 08 Mpa)
Concentrates
Dry at 60-70 °C under vacuum (0.08 Mpa)



(Solid yield at 4.5-5% with the content of Tanshinone IIA, a chemical marker/one of the active chemicals >2.0 %)

Fig 13.


Whole fruit
Add enough water (10-folds of raw materials) and macerate for 24 hours
and filter (100 mesh)
Solution
Residues
Dry under 60-70 °C for 8-12 hours
Pulverise into coarse powder and pass through a sieve (100mesh)
Coarse powder
Add enough 75% ethanol (5-folds of raw materials)
Reflux extraction
For 2 hours, separate the solution
Solution
Residue
Add 75% ethanol (5-folds of raw materials)
Reflux extraction
For 2 hours, separate the solution



(Solid yield at 4.5% with the content of Schisandrol A >2%)

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Received: 13.7.04.
Agent's Ref: P1658phy$040707
DCS

Your Reference:
Application No: GB0403708.1

The Patent Office
Patents Directorate

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http://www.patent.gov.uk

7 July 2004 PYN17

Dear Sirs

Patents Act 1977:
Combined Search and Examination Report under Sections 17 and 18(3)

Title: A botanical drug or dietary supplement

Latest date for reply: 20 February 2006

We have given your application a number GB0403708.1 which you should quote whenever you contact us about this application. The date of filing of the application is 19 February 2004.

I enclose a copy of my search and examination report and a copy of the documents referred to in my report.

Foreign language document

Only the abstract of the following foreign language document has been sent to you:

CN 1418637 A
CN 1183288 A

There will be some delay before I can obtain the source document and send it to you.

Non-patentable matter

Claims 46-48 relate to a method of treatment of the animal or human body and are therefore non-patentable under section 4(2) of the Patents Act 1977.

Opportunity to file amendments

By the latest date given above you should deal with the points raised in the report by filing amendments. These should be in the form of retyped pages filed in duplicate. However if you do not agree with any part of the report, then you should explain your reasons in a letter.

Important: you should avoid giving any additional technical information about the invention (such as a modification) either by way of amendment or in an accompanying letter, as this would prevent you from subsequently obtaining a patent based on this information.

Consequence of failing to reply

The application may be refused unless you reply to the report by the date set.

Further action

If after receiving your reply there are still points which need attention, I will contact you again. Should we still disagree, then the matter can be referred to a senior officer who will consider the issues afresh. You would have the opportunity, in this event, to come to the Office and present your opinion personally.

Publication

I estimate that soon after 12 July 2005 we will send your application for publication. At this time you will receive a letter confirming the exact date when the preparations for publication will be completed. This letter will also tell you the publication number and date of publication of your application.

Withdrawal

If you withdraw your application before we have sent it for publication, it will not be published. **WARNING** – after preparations for publication are complete it will NOT be possible to withdraw your application from publication.

Application abroad

If you wish to apply for a patent in a foreign country for your invention, you should do so before your application is published (or before 19 February 2005 if you will be using the present application as a priority document). If you do not, the publication of your application would invalidate a foreign application in most countries.

Further Information

The Patent Office has produced two explanatory booklets (**patents: essential reading** and **patents: application guide**) which provide important information about the patent system. If you do not have these booklets already, please contact our Central Enquiry Unit on 08459 500505 to request copies.

Correspondence

All correspondence should be addressed to the Comptroller.

While my e-mail address is provided above, you should note that the Office is unable to accept documents, such as amendments to the specification, transmitted by e-mail. Your official response to my report must therefore be delivered by post, fax or hand. However, you may use e-mail if you have any questions about the report or the processing of the application.

If you write to the Office less than 3 weeks before we are due to send your application for publication, please mark your letter prominently: "**URGENT - PUBLICATION IMMINENT**".

Yours faithfully

D. Cannon

Dave Cannon
Examiner

Your ref :		**Examiner :**	Dave Cannon
Application No:	GB0403708.1	**Tel :**	01633 814347
Applicant :	Phynova Limited	**Date of report :**	7 July 2004
Latest date for reply:	20 February 2006	Page 1/2	

Patents Act 1977
Examination Report under Section 18(3)

Non-patentable matter

1. Claims 46-48 relate to a method of treatment of the animal or human body, and are therefore non-patentable under section 4(2) of the Patents Act 1977. Consequently, the corresponding references in the description of invention to "methods of treatment" should be removed. Such references occur on page 7 lines11-13, and page 10 lines 5-8, 10-13, 18-22 and 24-27.

Clarity (Section 14(5)(b))

2. The use of the word "preferably" in claim 49 is ambiguous, and therefore the claim requires amending to more clearly define its scope.

Support (Section 14(5)(c))

3. The Prendergast decision [2000] RPC 446 outlines that for a claimed medical use of a substance or composition to be fully supported in an application, evidence is required in the description to illustrate the efficacy of said compound or substance for such a medical use. As such, there is only support in the description of invention and examples of the present application for a combination of the specific species *Silybum marianum, Astraglus membranaceus var mongholicus, Salvia miltiorrhiza, and Schisandra chinensis* (See example 2) for the treatment of Hepatitis C. Consequently, claims 1-5, 8-10, 16-30, 32-35, 45 and 49 will require amendment to limit them to these specific species.

4. There is no evidence in the description of invention or examples to support the use of the genus *Hedysarum* rather than *Astragalus* in a botanical drug or dietary supplement as outlined in claim 1. Consequent amendment to claims 1, 3, 7-10 and 49, and the description of invention on page 5 line 24, page 6 lines 6-7, page 10 line 32, and page 11 lines 6, 15 and 21, will be required to remove all reference to this species from the application.

5. In addition to paragraph 3 above, there is only support for the **fruit** extracts of *Silybum marianum,* and *Schisandra chinensis*, and the **root** extracts of *Astraglus membranaceus var mongholicus, and Salvia miltiorrhiza,* as outlined in example 2. As such, it is considered that claims 6 and 7 that relate to these aspects of the composition should be made an essential feature of claim 1.

[Examination Report contd.]

6. There is no support in the description of invention for the use of "defined extract fractions" in a botanical drug according to the present invention, as outlined in claim 14. The description of invention will consequently require amendment in this respect.

7. There is no support in the description of invention or examples for an extract of *Silybum* having a residual organic solvent content of no more than 0.01% hexane, as outlined in claim 18, part (v). As such, this embodiment should be removed from the claim.

8. Claim 39 is unsupported by the description of invention as there is no disclosure of a botanical drug according to the present invention containing a xanthan gum of such a specific molecular weight.

9. The description of invention is inconsistent with claim 40 which outlines the use of macrogol (polyethylene glycol) as a wetting agent in a botanical drug formulation according to the present invention. The description of invention on page 13, line 9 does not outline such compounds as suitable wetting agents, and as such will require amendment.

10. Claims 43 and 44 are not supported by the description of invention, as although in example 3 reference is made to the powdered drug being contained inside a sachet, no disclosure is made to other "dispensing containers" as outlined in claims 43 and 44.

Minor matters

11. On page 10 line 32 of the description of invention, and claim 8, a botanical drug or dietary substance composition is outlined in which the *Astragalus* or *Hedysarum* species is present in an amount from 20-95%. As the sum of the minimum quantities of the other species is greater than 5%, it is considered that the upper limit of this range is incorrect. Subsequent amendment is required to correct this error.

12. In examples 9-11 and figures 1-3 there is no explanation of the abbreviation BDS. It has been assumed that it is meant to mean 'botanical drug substance', however, on the first use of such an abbreviation its full meaning should also be given, and as such, appropriate amendment will be required.

13. The description on page 9, lines 12-19 is inconsistent with claims 23, 24 & 26, and examples 6 and 10 as it refers to the *Salvia* extract containing Tansihinon IIA rather than Tanshinone IIA. Amendment is required so that references to this compound are consistent.

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Application No:	GB0403708.1	**Examiner:**	Dave Cannon
Claims searched:	1-49	**Date of search:**	6 July 2004

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular reference
A	-	CN 1418637 A (WANG). See WPI and EPO abstracts.
A	-	CN 1183288 A (ZOU et al). See EPO abstract.
A	-	US 6043276 A (HAN MYUN K et al). See whole document.
A	-	JP 2001039868 A (CHO et al). See WPI and PAJ abstracts.

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC[W] :

A5B

Worldwide search of patent documents classified in the following areas of the IPC[07]

A61K; A61P

The following online and other databases have been used in the preparation of this search report

EPODOC, WPI, PAJ, TXTE, OPTICS, CAS-ONLINE, NAPRALERT

A BOTANICAL DRUG OR DIETARY SUPPLEMENT

TECHNICAL FIELD OF THE INVENTION

The present invention relates to a botanical drug or dietary supplement for use in the treatment of patients suffering from Hepatitis C virus infection. More particularly, it relates to a botanical drug consisting essentially of four botanical drug substances, optionally formulated with excipients, for use either in alleviating the symptoms of Hepatitis, particularly chronic Hepatitis C, and / or inhibiting the activity of the causative Hepatitis C virus.

BACKGROUND OF THE INVENTION

Chronic infection with the Hepatitis C virus (HCV) is common, affecting up to 1% of the UK population. It is well recognised that chronic HCV infection is associated with a wide variety of symptoms including fatigue, upper abdominal pain and dyspepsia, which lead to an overall reduction in the quality of life. Conventional therapy with pharmaceutical agents leads to an improvement in symptoms but is effective in only 40% of patients. There is thus a need for effective treatments that can reduce the symptoms associated with chronic Hepatitis C virus (HCV) infection and thereby improve the quality of life of a greater percentage of HCV patients.

DEFINITIONS

In the specification the following definitions, taken from the U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), August 2000 Guidance for Industry, Botanical Drug Products, are intended:

Active Constituent: The chemical constituent in a botanical raw material, drug substance, or drug product that is responsible for the intended pharmacological activity or therapeutic effect.

Botanical Product; Botanical: A finished, labelled product that contains vegetable matter, which may include plant materials (see below), algae, macroscopic fungi, or combinations of these. Depending in part on its intended use, a botanical product may be a food, drug, medical device, or cosmetic.

Botanical Drug Product; Botanical Drug: A botanical product that is intended for use as a drug; a drug product that is prepared from a botanical drug substance. Botanical drug products are available in a variety of dosage forms, such as solutions (e.g., teas), powders, tablets, capsules, elixirs, and topicals.

Botanical Drug Substance: A drug substance derived from one or more plants, algae, or macroscopic fungi. It is prepared from botanical raw materials by one or more of the following processes: pulverization, decoction, expression, aqueous extraction, ethanolic extraction, or other similar process. It may be available in a variety of physical forms, such as powder, paste, concentrated liquid, juice, gum, syrup, or oil. A botanical drug substance can be made from one or more botanical raw materials (see Single-Herb and Multi-Herb botanical drug substance or product). A botanical drug substance does not include a highly purified or chemically modified substance derived from natural sources.

Botanical Ingredient: A component of a botanical drug substance or product that originates from a botanical raw material.

Botanical Raw Material: Fresh or processed (e.g., cleaned, frozen, dried, or sliced) part of a single species of plant or a fresh or processed alga or macroscopic fungus.

Chromatographic Fingerprint: A chromatographic profile of a botanical raw material or drug substance that is matched qualitatively and quantitatively against that of a reference sample or standard to ensure the identity and quality of a batch and consistency from batch to batch.

Dietary Supplement: [A] product (other than tobacco) intended to supplement the diet that bears or contains one or more of the following dietary ingredients: (A) a vitamin; (B) a mineral; (C) an herb or other botanical; (D) an amino acid; (E) a dietary substance for use by man to supplement the diet by increasing the total dietary intake; or (F) a concentrate, metabolite, constituent, extract, or combination of any ingredient described in clause (A), (B), (C), (D), or (E); (2) means a product that (A) is intended for ingestion in a form described in section 411(c)(1)(B)(i) [of the FD&C Act]; or complies with section 411(c)(1)(B)(ii); is not represented for use as a conventional food or as a sole item of a meal or the diet; and is labeled as a dietary supplement; and (3) does (A) include an article that is approved as a new drug under section 505 or licensed as a biologic under section 351 of the Public Health Service Act (42 U.S.C. 262) and was, prior to such approval, certification, or license, marketed as a dietary

supplement or as a food unless [FDA] has issued a regulation, after notice and comment, finding that the article, when used as or in a dietary supplement under the conditions of use and dosages set forth in the labelling for such dietary supplement, is unlawful under section 402(f); and (B) not include (i) an article that is approved as a new drug under section 505, certified as an antibiotic under section 507, or licensed as a biologic under section 351 of the Public Health Service Act (42 U.S.C. 262), or (ii) an article authorized for investigation as a new drug, antibiotic, or biological for which substantial clinical investigations have been instituted and for which the existence of such investigations has been made public, which was not before such approval, certification, licensing, or authorization marketed as a dietary supplement or as a food unless [FDA], in [its] discretion, has issued a regulation, after notice and comment, finding that the article would be lawful under this Act_ (21 U.S.C. 321(ff)).

Dosage Form: A pharmaceutical product type, for example, tablet, capsule, solution, or cream, that contains a drug ingredient (substance) generally, but not necessarily, in association with excipients.

Drug: Means (A) articles recognized in the official United States Pharmacopeia, official Homeopathic Pharmacopeia of the United States, or official National Formulary, or any supplement to any of them; and (B) articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals; and (C) articles (other than food) intended to affect the structure or any function of the body of man or other animals; and (D) articles intended for use as a component of any articles specified in clause (A), (B), or (C). A food or dietary supplement for which a claim, subject to sections 403(r)(1)(B) and 403(r)(3) [of the FD&C Act] or sections 403(r)(1)(B) and (r)(5)(D), is made in accordance with the requirements of section 403(r) is not a drug solely because the label or the labeling contains such a claim. A food, dietary ingredient, or dietary supplement for which a truthful and not misleading statement is made in accordance with section 403(r)(6) is not a drug under clause (C) solely because the label or the labelling contains such a statement_ (21 U.S.C. 321(g)(1)).

Drug Substance: An active ingredient that is intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease or to affect the structure or any function of the human body (21 CFR 314.3(b)).

Drug Product: The dosage form in the final immediate packaging intended for marketing.

Food: The term *food* means (1) articles used for food or drink, (2) chewing gum, and (3) articles used for components of such articles (21 U.S.C. 321(f)).

Formulation: A formula that lists the components (or ingredients) and composition of the dosage form. The components and composition of a multi-herb botanical drug substance should be part of the total formulation.

Marker: A chemical constituent of a botanical raw material, drug substance, or drug product that is used for identification and/or quality control purposes, especially when the active constituents are not known or identified.

Multi-Herb (Botanical Drug) Substance or Product: A botanical drug substance or drug product that is derived from more than one botanical raw material, each of which is considered a botanical ingredient. A multi-herb botanical drug substance may be prepared by processing together two or more botanical raw materials, or by combining two or more single-herb botanical drug substances that have been individually processed from their corresponding raw materials. In the latter case, the individual single-herb botanical drug substances may be introduced simultaneously or at different stages during the manufacturing process of the dosage form.

Plant Material: A plant or plant part (e.g., bark, wood, leaves, stems, roots, flowers, fruits, seeds, berries, or parts thereof) as well as exudates.

Single-Herb (Botanical Drug) Substance or Product: A botanical drug substance or drug product that is derived from one botanical raw material. Therefore, a single-herb substance or product generally contains only one botanical ingredient.

In addition the terms:

Consisting essentially is intended to refer back only to the presence of the botanical raw materials and their derivatives and excludes the presence of e.g. excipients used in the formulation;

Treatment is intended to refer to both symptomatic relief and/ or activity against the causative factor.

In Traditional Chinese Medicine (TCM), HCV infection is regarded as causing the following pathological changes in the body:

- accumulation of toxin and heat in the blood;
- consumption of vital energy and body fluid;
- stagnation of blood; and
- injury of liver and spleen function.

In order to address these different aspects existing TCM plant based formulations for HCV treatment usually contain many ingredients, typically ten or more. For practical purposes it would clearly be desirable and advantageous to minimise the number of botanical ingredients or botanical drug substances without in any way compromising therapeutic efficacy.

Surprisingly the applicant has found that a combination of only four plant species demonstrates activity against Hepatitis C virus.

SUMMARY OF THE INVENTION

According to a first aspect of the present invention there is provided a botanical drug or dietary supplement, for the treatment of or for use in patients with Hepatitis C infection, consisting essentially of botanical raw materials, botanical drug substances or botanical ingredients from a species of each of the genera:

(a) *Silybum;*
(b) *Astragalus* or *Hedysarum;*
(c) *Salvia;* and
(d) *Schisandra.*

The preferred species of each of the four botanical raw materials is set out below:

The *Silybum* species is typically *Silybum marianum*. Preferably the plant material of the *Silybum* species which is used in the composition of the invention is the fruit. The fruit of *Silybum marianum* is known in TCM as Sui Fei Ji and in Western Europe as milk thistle fruit.

The *Astragalus* species: is typically *Astragalus membranaceus* var *mongholicus*. Preferably the plant material of the *Astragalus* species which is used is the root. The root of *Astragalus membranaceus* var *mongholicus* is known in TCM as Huang Qi and in Western Europe as Astragalus root. As alternatives to *Astragalus membranaceus* var *mongholicus*, another *Astragalus* species, *A. membranaceus* or a *Hedysarum* species may be used. The preferred *Hedysarum* species is *Hedysarum polybotyrs*. The root of *Hedysarum polybotyrs* is known in TCM as Hong Qi.

The *Salvia* species is typically *Salvia miltiorrhiza*. Preferably the plant material of the *Salvia* species which is used in the composition of the invention is the root. The root of *Salvia miltiorrhiza* is known in TCM as Dan Shen and in Western Europe as Chinese sage root. Alternatively *Salvia bowleyana* or *Salvia przewalskii* may be used.

The *Schisandra* species is typically *Schisandra chinensis*. Preferably the plant material of the *Schisandra* species which is used in the composition of the invention is the fruit. The fruit of *Schisandra chinensis* is known in TCM as Wu Wei Zi, and in Western Europe as Schisandra fruit. Alternatively *Schisandra sphenanthera* may be used.

In a preferred embodiment the plant species are:

a) *Silybum marianum;*
b) *Astragalus membranaceus* var *mongholicus;*
c) *Salvia miltiorrhiza*; and
d) *Schisandra chinensis.*

In alternative embodiments the *Astragalus membranaceus* var *mongholicus* may be substituted with *Astragalus membranaceus* or *Hedysarum polybotyrs*.

A particularly preferred composition of the invention comprises: Sui Fei Ji; Dan Shen; Wu Wei Zi; and Huang Qi.

Whilst in a favoured embodiment the invention takes the form of a botanical drug, comprising or consisting essentially of botanical drug substances of each of the four plant species in further

embodiments the botanical drug may comprise or consist essentially of botanical ingredients of each of the species. Where the product is a dietary supplement the four plant species may additionally be in the form of botanical raw materials.

In the case of a botanical drug there may be present, in addition to the botanical drug substances, pharmaceutically acceptable excipients.

In the case of a dietary supplement there may be present in addition to the botanical raw materials, botanical drug substances or botanical ingredients one or more dietetically acceptable excipients.

The present invention also provides a method of treatment or dietary supplementation which comprises administering to a human a composition of the invention in an amount sufficient to support healthy liver function and/ or relieve the symptoms of Hepatitis C viral infection and/ or to reduce viral load.

The plant materials may be employed in the composition of the invention in any suitable form. This may for instance be as crude plant material, which is either fresh or dried, or as an extract of fresh or dried plant material, i.e. a botanical drug substance. The extract is preferably a total plant extract defined with reference to one or more chemical markers although defined fractions and botanical ingredients may also be used. The extract, most usually a botanical drug substance, is typically dried and used in powder form, most preferably as a lyophilised extract.

When botanical drug substance is used it is preferably pulverized. In this embodiment the botanical drug substance is dried and ground to a powder. The resulting powder of the or each botanical drug substance is then conveniently mixed together to form a plant based composition of the invention in powder form. This powder can be administered directly, for instance by being dispersed in a liquid for human subjects to drink. Alternatively the powder can be processed into any other conventional dosage form such as capsules, tablets or granules. In a preferred embodiment the applicant has developed a suspension formulation which is suspendable in a relatively small volume of a cold liquid, such as water. Typically the suspension formulation can be suspended in less than 50ml, more typically less than 25 ml of water.

A botanical drug substance, for instance a total extract, may be prepared by any conventional technique known for the extraction of ingredients from botanical materials. These include solvent extraction including supercritical fluid extraction using a liquefied gas such as carbon dioxide. In one embodiment the extracts are ethanolic extracts, such as those obtained using 70% ethanol. The extracts are most preferably standardised extract, for instance a standardised total extract. The preferred standardised total extracts are pharmaceutical grade extracts.

An extract is typically prepared by immersing or macerating or refluxing fresh or dry plant material, for instance powdered dry plant material, in a suitable solvent; separating solid residue from the solution, removing the solvent from the solution; and recovering the resulting concentrates.

If desired a liquid extract may be dried before being formulated into a botanical drug or dietary supplement of the invention, for instance by spray drying or by freeze drying (lyophilisation). In that case the dried extract of one or more of the constituent plant species of the composition of the invention may be mixed with pulverized dried plant material of one or more of the other constituent plant species, to form a powder for direct administration to human subjects or for encapsulation or tabletting into unit dosage forms. Alternatively the extract may be used directly without prior drying.

The botanical raw materials or botanical drug substances or botanical ingredients may be combined together using any conventional technique that is suitable for ingredients of this type. When the botanical raw materials, drug substances or botanical ingredients are all in dry form they are conveniently mixed together, for instance by hand or by means of a mechanical mixer. A mixing procedure of this type may also be suitable if some, but not all, of the components of the plant based composition are in dry form.

The *Silybum marianum* is preferably employed in the form of a pharmaceutical grade extract that can be obtained commercially from, for example, an Italian manufacturer, Indena. The pharmaceutical grade *Silybum marianum* extract manufactured by Indena is standardized for silymarin content of no less than 30% weight percent by HPLC . The pharmaceutical grade extract must pass extensive safety and efficacy procedures. Preferably, when employed in the practice of the present invention the *Silybum marianum* extract has a minimum silymarin content of at least 30% by HPLC analysis.

The *Astragalus membranaceus* var *mongholicus* is preferably employed in the form of a pharmaceutical grade extract that can be obtained commercially from, for example, a Chinese manufacturer, the Institute of Medicinal Plant Development, Haiding District, Xibeiwang, Beijing 100094, China. Pharmaceutical grade *Astragalus membranaceus* var *mongholicus* extract manufactured in China is standardized for an Astragaloside IV content of about 0.4 weight percent. The pharmaceutical grade extract must pass extensive safety and efficacy procedures. Preferably, when employed in the practice of the present invention the *Astragalus membranaceus* var *mongholicus* extract has an Astragaloside IV content of from 0.1 to about 10 weight percentage. Preferably, the *Astragalus membranaceus* var *mongholicus* extract used in the present invention has a minimum Astragaloside IV content of at least 0.4 percent.

The *Salvia miltiorrhiza* is preferably employed in the form of a pharmaceutical grade extract that can be obtained commercially from, for example, a Chinese manufacturer, the Institute of Medicinal Plant Development, Haiding District, Xibeiwang, Beijing 100094, China. Pharmaceutical grade *Salvia miltiorrhiza* extract manufactured in China is standardized for a Tanshinon IIa content of about 1.5 weight percent. The pharmaceutical grade extract must pass extensive safety and efficacy procedures. Preferably, when employed in the practice of the present invention the *Salvia miltiorrhiza* extract has a Tanshinon IIa content of from 1.5 to about 50% weight percentage. Preferably, the *Salvia miltiorrhiza* extract used in the present invention has a minimum Tanshinon IIa content of at least 2.0 percent.

The *Schisandra chinensis* is preferably employed in the form of a pharmaceutical grade extract that can be obtained commercially from, for example, a Chinese manufacturer, the Institute of Medicinal Plant Development, Haiding District, Xibeiwang, Beijing 100094, China. Pharmaceutical grade *Schisandra chinensis* extract manufactured in China is standardized for a Schisandrol A content of no less than 2.0 weight percent. The pharmaceutical grade extract must pass extensive safety and efficacy procedures. Preferably, when employed in the practice of the present invention the *Schisandra chinensis* extract has a Schisandrol A content of from 1.0 to 50 weight percentage. Preferably, the *Schisandra chinensis* extract used in the present invention has a minimum Schisandrol A content of at least 2.0 weight percent.

The species of the present invention each support healthy liver function and in combination may be used to reduce or alleviate the symptoms associated with Hepatitis viral infection, especially Hepatitis C viral infection.

According to a second aspect of the present invention there is provided method of treating a patient to reduce or alleviate the symptoms of Hepatitis, particularly Hepatitis C, or to support healthy liver function comprising administering a botanical drug, or dietary supplement according to the first aspect of the present invention.

According to a third aspect of the invention there is provided the use of a botanical drug or dietary supplement according to the first aspect of the present invention in combination with another drug to reduce or alleviate the symptoms of Hepatitis, particularly Hepatitis C, or to support healthy liver function.

The another drug is preferably interferon and the composition of the invention may be provided simultaneously or sequentially with the interferon.

Thus, the invention also provides a method of treating, reducing or alleviating the symptoms of Hepatitis C in human subjects, which method comprises the administration thereto of a therapeutically effective amount of a botanical drug of the invention. The invention also provides a method of supporting healthy liver function in human subjects, which method comprises the administration thereto of a therapeutically effective amount of botanical drug of the invention.

The herbal composition may also be used to supplement the diet. The invention accordingly provides a method of dietary supplementation which comprises the administration to human subjects of a composition of the invention as described above in an amount effective to support healthy liver function.

The botanical drug or dietary supplement preferably contains each species in an amount, relative to the total weight of all of the botanical raw materials or botanical ingredients, as follows:

(a) *Silybum* spp. from 22-48%;

(b) *Astragalus* spp. or *Hedysarum* spp. from 20-95%;

(c) *Salvia* spp. from 13-48%; and

(d) *Schisandra* spp. from 2-19%.

More preferably still each species is present in an amount as follows:

(a) Silybum spp. from 30-40%;

(b) Astragalu spp. or *Hedysarum* spp. from 20-30%;

*(c) Salvia*spp. from 20-30%; and

(d) *Schisandra*spp. from 7.5-15%.

According to yet a further aspect of the present invention there is provided a botanical drug or dietary supplement, for the treatment of or for use in patients with Hepatitis C infection, comprising botanical raw materials, botanical drug substances or botanical ingredients from a species of each of the genera:

(a) Silybum;

(b) Astragalus or *Hedysarum;*

(c) Salvia; and

(d) Schisandra

in an amount by weight relative to the total weight of the botanical raw materials, botanical drug substance: botanical ingredients as follows:

(a) Silybum spp. no less than 22% and more preferably no less than 30%;

(b) Astragalus spp. or *Hedysarum* spp. no less than 20%

(c) Salvia spp. no less than 13% and more preferably no less than 20%; and

(d) Schisandra spp. no less than 2% and more preferably no less than 7.5%.

According to the present invention, a therapeutically effective amount of the compositions of the invention are amounts sufficient to reduce or alleviate the symptoms of HCV infection while minimizing harmful side effects. In one embodiment, the therapeutically effective amount is an amount sufficient to reduce or alleviate the symptoms of chronic Hepatitis C without causing harmful side effects. In another embodiment, the therapeutically effective amount is an amount sufficient to normalize or support healthy liver function without causing harmful side effects.

The dosage to be administered will vary and depend on the age, weight, sex and condition of the patient. Typical daily dosages of each of the plant based components (illustrated by way of example only with reference to the preferred species) are as follows (weights refer to a dry botanical raw material equivalent):

Silybum marianum:	2 – 15g
Astragalus membranaceus var *mongholicus*:	9 – 30g
Salvia miltiorrhiza:	9 – 15g
Schisandra chinensis:	1.5g – 6g

Dosages can be readily determined by one of ordinary skill in the art and can be readily formulated into the present supplemental and pharmaceutical compositions.

Botanical raw materials, botanical drug substances and botanical ingredients can be formulated into a medicament, dietary supplement or nutraceutical by conventional methods.

A nutraceutical is a food ingredient, food supplement or food product which is considered to provide a medical or health benefit, including the prevention and treatment of disease. In general a nutraceutical is specifically adapted to confer a particular health benefit on the consumer. A nutraceutical typically comprises a micronutrient such as a vitamin, mineral, herb or phytochemical at a higher level than would be found in a corresponding regular food product. That level is typically selected to optimise the intended health benefit of the nutraceutical when taken either as a single serving or as part of a diet regimen or course of nutritional therapy.

A botanical drug or dietary supplement of the present invention may be formulated into a medicament or dietary supplement by mixing with a dietetically or pharmaceutically acceptable carrier or excipient. Such a carrier or excipient may be a solvent, dispersion medium, coating, isotonic or absorption delaying agent, sweetener or the like. Suitable carriers may be prepared from a wide range of materials including, but not limited to, diluents, binders and adhesives, lubricants, disintegrants, colouring agents, bulking agents, flavouring agents, sweetening agents and miscellaneous materials such as buffers and adsorbents that may be needed in order to prepare a particular dosage form. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar

as any conventional media or agent is known to be incompatible with the plant based composition of the present invention, its use in the present compositions is contemplated.

For example, a solid oral forms may contain, together with the active components, diluents such as lactose, dextrose, saccharose, cellulose, corn starch or potato starch; lubricants such as silica, talc, stearic acid, magnesium or calcium stearate and/or polyethylene glycols; binding agents such as starches, arabic gums, gelatin, methylcellulose, carboxymethylcellulose, or polyvinyl pyrrolidone; disintegrating agents such as starch, alginic acid, alginates or sodium starch glycolate; effervescing mixtures; dyestuffs, sweeteners; wetting agents such as lecithin, polysorbates, lauryl sulphates. Such preparations may be manufactured in known manners, for example by means of mixing, granulating, tabletting, sugar coating, or film-coating processes.

Liquid dispersions for oral administration may include water solutions, tinctures, syrups, emulsions and suspensions. The syrups may contain as carrier, for example, saccharose or saccharose with glycerol and/or mannitol and/or sorbitol. In particular, a syrup for diabetic patients can contain as carriers only products, for example sorbitol, which do not metabolise to glucose or which only metabolise a very small amount to glucose. The suspensions and the emulsions may contain as carrier, for example, a natural gum, agar, sodium alginate, pectin, methylcellulose, carboxymethylcellulose or polyvinyl alcohol.

The botanical drug or dietary supplement of the present invention is also suitably formulated into granules or a powder. In this form it can be readily dispersed in water or other liquid such as tea or a soft drink for human patients to drink. It may also be encapsulated, tabletted or formulated with a physiologically acceptable vehicle into unit dosage forms. A unit dosage can comprise a therapeutically effective amount of the extract for a single daily administration, or it can be formulated into smaller quantities to provide for multiple doses in a day. The composition may thus, for instance, be formulated into tablets, capsules, syrups, elixirs, enteral formulations or any other orally administrable form. Examples of physiologically acceptable carriers include water, oil, emulsions, alcohol or any other suitable material

The present invention will be further illustrated, by way of Example, only with reference to the following formulations and data in which:

Fig 1 is a TCL picture of the BDS of *Astragalus membranaceus* var *mongholicus*;

Fig 2 is a TCL picture of the BDS of *Salvia miltiorrhiza*;

Fig 3 is a TCL picture of the BDS of *Schisandra chinensis*.

Fig 4 is a HPLC chromatogram of the BDS of *Astragalus membranaceus*;

Fig 5 is a HPLC chromatogram of Astragaloside (a marker of *Astragalus membranaceus* var *mongholicus*)

Fig 6 is a HPLC chromatogram of the BDS of *Salvia miltiorrhiza*;

Fig 7 is a HPLC chromatogram of Tanoshone-IIA (a marker of *Salvia miltiorrhiza)*

Fig 8 is a HPLC chromatogram of the BDS of *Schisandra chinensis*;

Fig 9 is a HPLC chromatogram of Schisandrin (a marker of *Schisandra chinensis);*

Fig 10 is a flow chart showing the manufacture process for producing a botanical drug substance from *Silybum* spp.;

Fig 11 is a flow chart showing the manufacture process for producing a botanical drug substance from *Astragalus* spp.;

Fig 12 is a flow chart showing the manufacture process for producing a botanical drug substance from *Salvia* spp.; and

Fig 13 is a flow chart showing the manufacture process for producing a botanical drug substance from *Schisandra* spp.

DETAILED DESCRIPTION

EXAMPLE 1:

Preparation of botanical drug from botanical drug substances

Standardised extracts of *Silybum marianum* (fruit), *Salvia miltirrhiza* (root), *Schisandra chinensis* (fruit), and *Astragalus membranaceus* var *mongholicus* (root) were made separately using extraction procedures designed specifically for each herb in order to achieve the desired therapeutic potency of the extracts. The extracts were dried and the resulting dry powdered extracts mixed in the proportions shown below (the weights are given both for the extracts and as an equivalent by weight of dry botanical raw material).

(a) *Silybum marianum;* from 0.200g to 0.250g (equivalent to 12g to 15g of botanical raw material),

(b) *Astragalus membranaceus* var *mongholicus;* 0.585g to 1.95g (equivalent to 9g to 30g of botanical raw material)

(c) *Salvia miltirrhiza;* 0.225g to 0.375g (equivalent to 9g to 15g f botanical raw material) and

(d) *Schisandra chinensis;* 0.150g to 0.600g (equivalent to 1.5g to 6g of botanical raw material).

EXAMPLE 2:

Formulation into a suspension mixture

The spray-dried botanical drug substances of Example 1 were formulated into a suspension dosage form by mixing the spray-dried botanical drug substances with:

a) one or more gellants or thickeners comprising at least one xanthum gum having a particle size distribution such that 100% by weight of the particles pass a 60 mesh sieve, 95% by weight of the particles pass a 80 mesh sieve and 70% by weight of the particles pass a 200 mesh sieve,

b) one or more fillers; and

c) one or more wetting agents and or surfactants.

The resulting formulation, referred to as the PYN17 suspension powder mixture, contained the following:

Composition:		per sachet
Active ingredients:		
Milk Thistle Fruit dry extract	:	0,200 g
Chinese Sage Root dry extract	:	0,225 g
Schisandra Fruit dry extract	:	0,400 g
Astralagus Root dry extract	:	0,585 g
Excipients:		
Macrogol 6000 powder	:	0,600 g
Ferwogel 30.385	:	0,070g

Mannitol EZ	:	0,160g
Aerosil 200	:	0,050g
Aspartame	:	0,050 g
Caramel powder	:	0,100 g
Peppermint powder aroma	:	0,060g

EXAMPLE 3:

Activity of PYN17 suspension powder mixture

A sachet of the suspension powder was re-suspended in 2.5ml water and further diluted 1 in 7. The incompletely dissolved suspension was filtered and the soluble fraction tested.

10 µl of solution was tested in 100µl culture of cells at a concentration of 1/70.
Concentrations of 1/350 and 1/1750 were also used to determine toxicity.

To test toxicity the cells were cultured with Replicon cells for 72 hours, and tritiated thymidine was added 18 hours prior to harvesting.

Results:

Tritiated thymidine incorporation.

Dilution PYN-17	Well 1 cpm	Well 2 cpm	Well 3 cpm	Well 4 cpm	Well 5 cpm	Mean cpm
1/70	18	24	65	51	77	
1/350	41010	32432	34719	30311	32371	34169
1/1750	36210	28315	32424	38230	39815	34999
0	31609	35373	36199	36281	36210	35134

Inhibition of replication measured by expression of Renilla luciferase.

The 1/70 dilution was toxic to the cells (as under the microscope the cells were dead).
This dilution was not used in the Replicon assay and a further lower dilution was used.

Dilution PYN17	Well 1 luciferase activity	Well 2	Well 3	Well 4	Well 5	Mean luciferase activity	+/- SD
1/350	531292	234958	614669	479425	725350	517139	183108
1/1750	594920	972891	889324	595922	-	763264	196789
1/8750	880338	1005370	608077	644105	806756	788929	165228

0	1139829	870757	820645	724027	-	888815	178079

CONCLUSION

At a 1/350 dilution an inhibition of 41.8 % was noted indicating activity against Hepatitis C virus.

The results may be slightly skewed by one very low result (well 2).

The control (no suspension powder) may also be skewed by the one high result (well 1).

At 1/350 the mean without the low result was 587684

The control without the high result (well 1) was 805143

Excluding the single high and low results the % inhibition was 27%.

EXAMPLES 4 -7

These illustrate the extraction methods used in the preparation of the botanical drug substances used in the botanical drug of the invention.

Example 4

Preparation of a botanical drug substance from a *Silybum* spp.

Referring to Fig 10 there is illustrated a process for producing a botanical drug substance of a *Silybum* spp. The fruits are prepared for extraction, undergo an extraction, the resulting solution is filtered, and concentrated. The concentrated purified extract then undergoes a further clean up process in which purified product is precipitated, filtered and the filtrate dried and ground for packing. Such a product can be obtained from Indena SpA.

Example 5

Preparation of a botanical drug substance from a *Astragalus* spp.

(The preparation of a botanical drug substance from a *Hedysarum* spp. is equivalent)

Referring to Fig 11 *Astragalus* spp. root material is dried in an oven at 60^{O}C for 3 hours, pulverised into a coarse powder, passed through a sieve (10 mesh) and subjected to extraction as per the flow chart. The extraction process is an ethanolic extraction. The concentrate obtained is re-dissolved in ethanol, any precipitate removed and the product concentrated and dried. The method yields a solid content in excess of 10% with an Astragaloside content of greater than 0.4%.

Example 6

Preparation of a botanical drug substance from a *Salvia* spp.

Referring to Fig 12 the *Salvia* spp. root material is dried in an oven at 60°C for 3 hours, pulverised into a coarse powder, passed through a sieve (10 mesh) and subjected to extraction as per the flow chart. The extraction process is an ethanolic extraction and the resulting concentrate is dried. The method yields a solid content in excess of 4% with a Tanshinone IIA content of greater than 1.5%.

Example 7

Preparation of a botanical drug substance from a *Schisandra* spp.

Referring to Fig 13 the *Salvia* spp. fruit is macerated in water and filtered. The filtrate residues are dried, powdered and subjected to an ethanolic extraction, and the resulting concentrate is dried. The method yields a solid content in excess of 4% with a Schisandrol A content of greater than 2%.

EXAMPLES 8-11

A botanical drug substance obtained from the sources identified, and by the methods described was subject to analysis and the results are given below:

EXAMPLE 8

The botanical drug substance from a *Silybum* spp. was shown by analysis to have the following characteristics

DETERMINATION	RESULTS	SPECIFICATIONS
SPECTROPHOTOMETRIC CONTENTS (as silymarin, calculated as silybin, according to DAB 10	70.3	>= 65.0
HPLC CONTENTS As sum of silybin and isosilybin	88.8	>= 80.0
CHARACTERS Brownish-yellow powder	Complies	Complies
SOLUBLE SUBSTANCES In pentane	0.25	<= 0.5
HPLC IDENTIFICATION	Complies	Complies
LOSS ON DRYING (T= 60°C, in vacuum, t= 3h)	0,0	<= 5.0
SULPHATED ASH According to Ph. Eur.	0.3%	<= 1.0
HEAVY METALS According to Ph. Eur. Method A	Complies	<= 100:0
RESIDUAL ORGANIC SOLVENTS Ethanol	0.4	<= 1.0
[illegible] state	< 0.0008	<= 0.01
	Complies	<= 0.01
MICROBIOLOGICAL CONTROL According to Ph. Eur. MESOPHILIC BACTERIA Maximum limit of acceptance: 1000 cfu/g M/0118	< 1000.0	<= 1000.0
YEAST Maximum limit of acceptance: 100 cfu/g M/0118	< 100.0	<= 100.0
ENTEROBACTERIA TM/0015 and TM/0075	< 100.0	<= 100.0
STAPHYLOCOCCUS AUREUS, SALMONELLA TM/0008, TM/0009, TM/0017 and TM/0078	Absent	Absent
ESCHERICHIA COLI, PSEUDOMONAS AERUGINOSA TM/0010, TM/0011, TM/0016 and TM/0075	Absent	Absent

EXAMPLE 9

The botanical drug substance from the *Astragalus* spp. was shown by analysis to have the following characteristics:

A) Certificate of Analysis

Product Name: Astragalus Root Extract (*Astragalus membranaceus* var *mongholicus*)

Batch Number: AMR-200201PE

TESTS	SPECIFICATION	RESULT
Appearance	Pale yellow colour	Pass
Loss on Drying:	<5% (CP)	2.65%
Particle Size:	80 mesh	Pass
Total Ash	<5.0%	0.14%
Heavy Metals: Lead	<5ppm	0.55
Mercury	<1ppm	0.84
Arsenic	<1ppm	0.61
Cadmium	<0.5ppm	0.21
Acid Insoluble Ash	<2.0%	0.026%
Microbial Total viable aerobic count:	$< 10^3$ cfu/g	80
Fungal & Yeast:	$< 10^2$ cfu/g	10
Escherichia coli:	Absent in 10g	Absent
Salmonella spp.:	Absent in 10g	Absent
Content Assay :	Astragaloside IV >0.4%	0.44%

B) Chemical Analysis

Name of the Product: Astragalus Root Extract (*Astragalus membranaceus* var *mongholicus)*

Batch Number: AMR-200201PE

Chemical Analysis:

i) TLC Fingerprint: See Fig 1 which is a TLC picture of the BDS of *Astragalus membranaceus* var *mongholicus*. The left is the BDS sample and the right the standard reference chemical Astragaloside IV

Preparation of test solutions:

Add 40ml of methanol to 1g of powder extract, shake well and filter. Apply the filtrates to a prepared neutral aluminium oxide column, then follow the method described in Chinese Pharmacopoeia (English Edition, 2000), Page 161, Identification (2),

Reference solution: Dissolve chemical reference standard (CRS) Astragaloside IV in methanol to produce a 1mg/1ml reference solution.

Loadings: Load 2µl of the test solution and 2µl of the reference solution, respectively, on foil-backed Silica gel F_{254} plate (Merck).

Developing solvent system: chloroform: methanol: water (13: 7: 2) (Lower layer)

Developing: Add mixed developing solution to a TLC tank and stand for 15 Minute for equilibrium. Put the TLC plate in and develop for 7.5 cm.

Detection:
When sprayed with 10% of sulphuric acid in ethanol and heated at 105°C a brown spot is obtained in TLC chromatogram of the test solution corresponds in position and colour to the spot of the reference solution. Observe the developed TLC plate under $UV365_{nm}$ light, both reference chemical Astragaloside IVand test solution showed a orange yellow spot at Rf 0.49,

ii) HPLC analysis

Equipment: Waters HPLC System, LC 600 pump and UV detector (Model 486).

Column: Spherisorb S100Ds1, 25cm x 4.6mm

Column temperature: 25 °C

Flow rate: 1.0ml/min

Detection wavelength: $UV200_{nm}$

Mobile phase: acetonitrile: water (1: 2)

Preparation of CRS solution: Dissolve 2 mg of Astragaloside IV in mobile phase solution in a 10ml volumetric flask.

Preparation of test solutions:
Weigh accurately 1.0g of powder extract, add 50ml of 2% KOH in methanol, heat and reflux on water bath for 1 hour and filter. Repeat the procedure for three times. Combine the filtrates and recover the solvent. Add 25ml of water to dissolve the residue, wash with 50ml of ether. To the aqueous solution, extract with 25ml of n-butanol (saturated in water) for three times. Combine butanol solution, wash twice with 25ml of water, respectively, then wash with 25ml of potassium dihydrogen phosphate, recover the solvent. Add accurately 10ml of mobile phase solution to the residue shake well, filter through milipore (0.45 µm) as test solution.

Quantity of injection: Inject 20 µl of CRS solution and 20 µl of test solution, respectively.

Result: See chromatograms in Figs 4 and 5. Fig 4 (the BDS) shows at least 10 clearly identifiable peaks including Astragaloside IV at a retention time of about 20 minutes. The area under the graph indicates a presence of at least 0.4% by weight of Astragaloside IV. The Fig 5 chromatogram is a control with the marker alone.

Specifications for Astragaloside IV content (% w/w)	Result (% w/w)
>0.4	0.44

EXAMPLE 10

The botanical drug substance from the *Salvia* spp. was shown by analysis to have the following characteristics:

A) Certificate of Analysis

Product Name: Salvia Miltiorrhiza Root Extract (*salvia miltiorrhiza*)

Batch Number: SMR-200201PE

TESTS	SPECIFICATION	RESULT
Appearance	Dark red colour	Pass
Loss on Drying:	<5% (CP)	3.24%
Particle Size:	80 mesh	Pass
Total Ash	<5.0%	0.38%
Acid Insoluble Ash	<2.0%	0.04%
Heavy Metals: Lead	<5ppm	0.65
Mercury	<1ppm	0.14
Arsenic	<1ppm	0.62
Cadmium	<0.5ppm	0.38
Microbial Total viable aerobic count:	$< 10^3$ cfu/g	100
Fungal & Yeast:	$< 10^2$ cfu/g	20
Escherichia coli:	Absent in 10g	Absent
Salmonella spp.:	Absent in 10g	Absent
Content Assay :	Tanshinone$\square_A$> 1.5%	1.98%

B) Chemical Analysis

Name of the Product: Salvia Miltiorrhiza Root Extract (*Salvia miltiorrhiza*)

Batch Number: SMR-200201PE

Chemical Analysis:

i) TLC Fingerprints: See Fig 2 which is a TLC picture of the BDS of *Salvia miltiorrhiza*. The left is the BDS sample and the right the standard reference chemical Tanshinone IIA

Preparation of Test solutions: Add 1ml of ethyl acetate to 100mg of powder extract.

Reference solution: Dissolve chemical reference standard (CRS) Tanshinone II_A in ethyl acetate to produce a 2mg/1ml reference solution.

Loadings: Load 5µl of the test solution and 5µl of the reference solution, respectively, on foil-backed Silica gel plate (Merck).

Developing solvent system: benzene: ethyl acetate (19: 1)

Developing: Add mixed developing solution to a TLC tank and stand for 15 Minute for equilibrium. Put the TLC plate in and develop for 7.5 cm.

Detection: Dry the developed plate in air, a dark red spot obtained in TLC chromatogram of the test solution corresponds in position and colour to the spot of the reference solution at Rf 0.46.

ii) HPLC analysis

Equipment: Waters HPLC System, LC 600 pump and UV detector (Model 486).

Column: Spherisorb S100Ds1, 25cm x 4.6mm

Column temperature: 25 ^{o}C

Flow rate: 1.0ml/min

Detection wavelength: $UV270_{nm}$

Mobile phase: Methanol: Water (15: 5)

Preparation of CRS solution: Weight accurately 10 mg of Tanshinone IIA to a 50ml amber volumetric flask and dissolve with methanol to the volume. Accurately measure 2ml to a 25ml amber volumetric flask and add methanol to the volume.

Preparation of test solutions: Weigh accurately 30mg of powder extract to a 25ml volumetric flask, add 18ml of methanol and treat under ultrasonic for 5 minutes, then add methanol to the volume.

Quantity of injection: Inject 5µl of CRS solution and 5 µl of test solution, respectively.

Result: See chromatograms in Figs 6 and 7. Fig 6 (the BDS) shows at least 6 identifiable peaks including Tanshinone IIA at a retention time of about 28/29 minutes. The area under the graph indicates a presence of at least 1.5% by weight of Tanshinone IIA. The Fig 7 chromatogram is a control with the marker alone.

Specifications for Tanshinone II_A content (% w/w)	Result (% w/w)
>1.5	1.98

EXAMPLE 11

The botanical drug substance from the *Schisandra* spp. was shown by analysis to have the following characteristics:

A) Certificate of Analysis

Product Name: Schisandra Fruit Extract (*Schisandra chinensis*)

Batch Number: SCF-200201PE

TESTS	SPECIFICATION	RESULT
Appearance	Brownish red colour	Pass
Loss on Drying:	<5% (CP)	4.5%
Particle Size:	80 mesh	Pass
Total Ash	<5.0%	0.25%
Acid Insoluble Ash	<2.0%	0.06%
Heavy Metals: Lead	<5ppm	0.45
Mercury	<1ppm	0.47
Arsenic	<1ppm	0.74
Cadmium	<0.5ppm	0.36
Microbial Total viable aerobic count:	$< 10^3$ cfu/g	90
Fungal & Yeast:	$< 10^2$ cfu/g	10
Escherichia coli:	Absent in 10g	Absent
Salmonella spp.:	Absent in 10g	Absent
Content Assay :	Schizandrol A >2.0%	2.4%

B) Chemical Analysis

Name of the Product: Schisandra Fruit Extract (*Schisandra chinensis)*

Batch Number: SCF-200201PE

Chemical Analysis:

i) TLC Fingerprints: See Fig 3 which is a TLC picture of the BDS of *Schisandra chinensis*. The left is the BDS sample and the right the standard reference chemical Schisandrin A

Preparation of Test solutions:
Add 20ml of chloroform to 0.5g of powder extract, ultrasonicate for 10 minutes and filter. Evaporate the filtrates to dryness and dissolve the residue in 1ml of chloroform as test solution.

Reference solution: Dissolve chemical reference standard (CRS) Schizandrol A in chloroform to produce a 1mg/1ml reference solution.

Loadings: Load 2µl of the test solution and 2µl of the reference solution, respectively, on foil-backed Silica gel F_{254} plate (Merck).

Developing solvent system: Petroleum ether (30-60^{O} C): ethyl formate: formic Acid (15:5:1) (upper layer)

Developing: Add mixed developing solution to a TLC tank and stand for 15 minute for equilibrium. Put the TLC plate in and develop for 7.5 cm.

Detection: Dry the developed plate in air, observe the plate under UV 254nm, a dark spot obtained in TLC chromatogram of the test solution corresponds in position and colour to the spot of the reference solution at Rf 0.14.

ii) HPLC analysis

Equipment: Waters HPLC System, LC 600 pump and UV detector (Model 2487).

Column: Spherisorb S100Ds1, 25cm x 4.6mm

Column temperature: 25 ^{O}C

Flow rate: 1.0ml/min

Detection wavelength: $UV250_{nm}$

Mobile phase: Methanol: Water (13: 7)

Preparation of CRS solution: Weight accurately 15 mg of Schizandrol A to a 50ml volumetric flask and dissolve with methanol to the volume to produce a solution with 0.3mg Schizandrol A / per ml.

Preparation of test solutions: Place 0.25g of raw material powder (Trough No.3 sieve) into a volumetric flask, add 18ml of methanol and ultrasonicate (power 250w, frequency 20 kHz) for 20 minutes. Add methanol to the volume, mix well and filter.

Quantity of injection: Inject 10µl of CRS solution and 10 µl of test solution, respectively.

Result: See chromatograms in Figs 8 and 9. Fig 8 (the BDS) shows at least 6 identifiable peaks including Schizandrol A at a retention time of about 14/15 minutes. The area under the graph indicates a presence of at least 2% by weight of Schizandrol A. The Fig 9 chromatogram is a control with the marker alone.

Specifications for Schizandrol A content (% w/w)	Result (% w/w)
>2.0	2.4

CLAIMS

1. A botanical drug or dietary supplement, for the treatment of or for use in patients with Hepatitis C infection, consisting essentially of botanical raw materials, botanical drug substances or botanical ingredients from a species of each of the genera:

   (a) Silybum;

   (b) Astragalus or *Hedysarum;*

   (c) Salvia; and

   (d) Schisandra.

2. A botanical drug or dietary supplement as claimed in claim 1 wherein the species of *Silybum* is *Silybum marianum.*

3. A botanical drug or dietary supplement as claimed in any of the preceding claims wherein the species of *Astragalus* or *Hedysarum* is *Astragalus membranaceus* var *mongholicus* or *Hedysarum polybotrys.*

4. A botanical drug or dietary supplement as claimed in any of the preceding claims wherein the species of *Salvia* is *Salvia miltiorrhiza, Salvia bowleyana* or *Salvia przewalskii.*

5. A botanical drug or dietary supplement as claimed in any of the preceding claims wherein the species of *Schisandra* is *Schisandra chinensis or Schisandra sphenanthera* .

6. A botanical drug or dietary supplement as claimed in any of the preceding claims wherein the botanical raw material of the *Silybum* and *Schisandra* species is a fruit.

7. A botanical drug or dietary supplement as claimed in any of the preceding claims wherein the botanical raw material of the *Salvia* and *Astragalus* or *Hedysarum* species is root material.

8. A botanical drug or dietary supplement as claimed in any of the preceding claims wherein each species is present in an amount, relative to the total weight of all of the botanical raw materials, botanical drug substances or botanical ingredients, as follows:

   a) Silybum spp. from 22-48%;

   b) Astragalus spp. or *Hedysarum* spp. from 20-95%;

   c) Salvia spp. from 13-48%; and

   d) Schisandra spp. from 2-19%.

9. A botanical drug or dietary supplement as claimed in claim 8 wherein each species is present in an amount as follows:

   (a) Silybum spp. from 30-40%;

(b) *Astragalus* or *Hedysarum* spp. from 20-30%;

(c) *Salvia* spp. from 20-30%; and

(d) *Schisandra* spp. from 7.5-15%.

10. A botanical drug or dietary supplement as claimed in claim 8 or 9 wherein each species is present in an amount as follows:

(a) *Silybum* spp. 35.3% plus or minus 10%;

(b) *Astragalus* or *Hedysarum* spp. 26.5% plus or minus 10%;

(c) *Salvia* spp. 26.5% plus or minus 10%; and

(d) *Schisandra* spp. 11.7% plus or minus 10%.

11. A botanical drug as claimed in any of the preceding claims which consists essentially of botanical drug substances.

12. A botanical drug as claimed in claim 11 further comprising excipients.

13. A botanical drug as claimed in claim 11 wherein the botanical drug substances comprise total extracts derived from each of the botanical raw materials.

14. A botanical drug as claimed in claim 11 wherein the botanical drug substances comprise one or more defined extract fractions derived from each of the botanical raw materials.

15. A botanical drug as claimed in any of claims 11 to 14 in which the botanical drug substances are standardised extracts.

16. A botanical drug as claimed in claim 15 wherein the botanical drug substance from the *Silybum* spp. is standardised against a marker of silybin.

17. A botanical drug as claimed in claim 15 wherein the botanical drug substance from the *Silybum* spp. comprises at least 30% by weight silybin and isosilybin when calculated by HPLC method.

18. A botanical drug as claimed in any of claims 15 to 17 wherein the standardised extract of the *Silybum* spp. is a brownish yellow powder which is or has:

(i) no less than 30% silybin by HPLC;

(ii) no more than 0.5% soluble in pentane;

(iii) a sulphated ash content of no more than 1%;

(iv) a heavy metal content of no more than 100ppm;

(v) a residual organic solvent content of no more than 1% ethanol, no more than 0.01% ethyl acetate and no more than 0.01% hexane;

(vi) a bacterial content of no more than 1000 cfu/g; and

(vii) a fungal content of no more than 100cfu/g.

19. A botanical drug as claimed in claim 15 wherein the botanical drug substance from the *Astragalus* spp. is standardised against a marker of Astragaloside IV.

20. A botanical drug as claimed in claim 19 wherein the botanical drug substance from the *Astragalus* spp. comprises at least 0.4% by (weight) Astragaloside IV as calculated by HPLC method.

21. A botanical drug as claimed in either claim 19 or 20 wherein the botanical drug substance from the *Astragalus* spp. has a TLC chromatographic fingerprint substantially as illustrated in Fig 1 or a HPLC fingerprint substantially as illustrated in Fig 4.

22. A botanical drug as claimed in any of claims 19 to 21 wherein the standardised extract of *Astragalus* spp. is a pale yellow powder which is or has:

(i) no less than 0.4% Astragaloside IV;

(ii) a total ash content of no more than 5%;

iii) an acid insoluble ash content of no more than 2%; and

iv) a microbial total viable aerobic count of no more than of 1000 cfu/g.

23. A botanical drug as claimed in claim15 wherein the botanical drug substance from the *Salvia* spp. is standardised against a marker of Tanshinone II A.

24. A botanical drug as claimed in claim 23 wherein the botanical drug substance from the *Salvia* spp. comprises at least 1.5% by (weight) of Tanshinone IIA as calculated by HPLC method

25. A botanical drug as claimed in either claim 23 or 24 wherein the botanical drug substance from the *Salvia* spp. has a TLC chromatographic fingerprint substantially as illustrated in Fig 2 or a HPLC fingerprint substantially as illustrated in Fig 6.

26. A botanical drug as claimed in any of claims 23 to 25 wherein the standardised extract of the *Salvia* spp. is a dark red powder which is or has:

(i) no less than 1.5% Tanshinone IIA by HPLC;

(ii) a total ash content of no more than 5%;

iii) an acid insoluble ash content of no more than 2%; and

iv) a microbial total viable aerobic count of no more than of 1000 cfu/g.

27. A botanical drug as claimed in claim15 wherein botanical drug substance from the *Schisandra* spp. is standardised against a marker of Schizandrol A.

28. A botanical drug as claimed in claim 27 wherein the botanical drug substance from the *Schisandra* spp. comprises at least 2.0% by weight Schizandrol A by HPLC method.

29. A botanical drug substance, as claimed in either claim 27 or 28 wherein the botanical drug substance from the *Schisandra* spp. has a TLC chromatographic fingerprint substantially as illustrated in Fig 3 or a HPLC fingerprint substantially as illustrated in Fig 8.

30. A botanical drug substance as claimed in either claim 28 or 29 wherein the standardised extract of *Schisandra* spp. *is* a brownish red powder which is or has:

(i) no less than 2.0 % Schizandrol A;

(ii) a total ash content of no more than 5%;

iii) an acid insoluble ash content of no more than 2%; and

iv) a microbial total viable aerobic count of no more than of 1000 cfu/g.

31. A botanical drug as claimed in any of claims 15 – 30 wherein each standardised extract is a dried ethanolic extract.

32. A botanical drug as claimed in any of claims 15 – 31 wherein the *Silybum* spp. is extracted according to a process substantially as illustrated in Fig 10.

33. A botanical drug as claimed in any of claims 15 – 31 wherein the *Astragalus* spp. is extracted according to a process substantially as illustrated in Fig 11.

34. A botanical drug as claimed in any of claims 15 – 31 wherein the:

Salvia spp. is extracted according to a process substantially as illustrated in Fig 12.

35. A botanical drug as claimed in any of claims 15 – 31 wherein the *Schisandra* spp. is extracted according to the process substantially as illustrated in Fig 13..

36. A botanical drug as claimed in any of claims 15 – 35 which is provided in a unit dosage form.

37. A botanical drug as claimed in claims 36 which is a suspension powder mixture.

38. A botanical drug as claimed in claims 37 further comprising as excipients:

a) one or more gellants or thickeners comprising at least one xanthum gum having a particle size distribution such that 100% by weight of the particles pass a 60 mesh sieve, 95% by weight of the particles pass a 80 mesh sieve and 70% by weight of the particles pass a 200 mesh sieve,

b) one or more fillers; and

c) one or more wetting agents and or surfactants.

39. A botanical drug as claimed in claims 38 wherein the xanthan gum has a molecular weight of from 3.5 to 4.0 $x10^6$.

40. A botanical drug as claimed in claims 38 wherein the wetting agent is a polyethylene glycol or macrogol.

41. A botanical drug as claimed in any of claims 36 to 40 further comprising one or more of a disintegrating agent, a lubricant, a sweetening agent, a flavouring agent and a viscosifying agent.

42. A botanical drug as claimed in any of claims 36 to 41 which is packaged in a sachet.

43. A botanical drug as claimed in any of claims 36 to 42 which is packaged with a dispensing container.

44. A botanical drug as claimed in claim 43 wherein the dispensing container has a sealable lid.

45. A botanical drug as claimed in any of claims 15 to 45 comprising in a unit dose:

i) 0.200g to 0.250g of a botanical drug substance from a *Silybum* spp. (equivalent to 12g to 15g of botanical raw material);

ii) 0.585g to 1.95g of a botanical drug substance from a *Astragalus* spp. (equivalent to 9g to 30g of botanical raw material) ;

iii) 0.225g to 0.375g of a botanical drug substance from a *Salvia* spp. (equivalent to 9g to 15g f botanical raw material) and

iv) 0.150g to 0.600g of a botanical drug substance from a *Schisandra* spp. (equivalent to 1.5g to 6g of botanical raw material).

46. A method of treating a patient to reduce or alleviate the symptoms of Hepatitis, particularly Hepatitis C, or to support healthy liver function comprising administering to a patient a botanical drug or dietary supplement as claimed in any of claims 1-10 or a botanical drug as claimed in any of claims 11 to 45.

47. The use of a botanical drug or dietary supplement as claimed in any of claims 1-10 or a botanical drug as claimed in any of claims 11 to 45 in combination with another drug to reduce or alleviate the symptoms of Hepatitis, particularly Hepatitis C, or to support healthy liver function.

48. The use as claimed in claim 47 wherein the another drug is interferon.

49. A botanical drug or dietary supplement, for the treatment of or for use in patients with Hepatitis C infection, comprising botanical raw materials, botanical drug substances or botanical ingredients from a species of each of the genera:

(a) Silybum;

(b) *Astragalus* or *Hedysarum;*

(c) *Salvia;* and

(d) *Schisandra*

in an amount by weight relative to the total weight of the botanical raw materials, botanical drug substances or botanical ingredients as follows:

(a) Silybum spp. no less than 22% and more preferably no less than 30%;

(b) Astragalus or *Hedysarum* spp.no less than 20% ;

(c) Salvia spp. no less than 13% and more preferably no less than 20%; and

(d) Schisandra spp. no less than 2% and more preferably no less than 7.5%.

Application No:	GB0403708.1	**Examiner:**	Dave Cannon
Claims searched:	1-49	**Date of search:**	6 July 2004

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular reference
A	-	CN 1418637 A (WANG). See WPI and EPO abstracts.
A	-	CN 1183288 A (ZOU et al). See EPO abstract.
A	-	US 6043276 A (HAN MYUN K et al). See whole document.
A	-	JP 2001039868 A (CHO et al). See WPI and PAJ abstracts.

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category	P	Document published on or after the declared priority date but before the filing date of this invention
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC^{W} :

A5B

Worldwide search of patent documents classified in the following areas of the IPC^{07}

A61K; A61P

The following online and other databases have been used in the preparation of this search report

EPODOC, WPI, PAJ, TXTE, OPTICS, CAS-ONLINE, NAPRALERT